Received: 17 February 2017 | Revised: 7 April 2017 | Accepted: 2 May 2017

DOI: 10.1002/med.21452

WILEY

REVIEW ARTICLE

Tumor angiogenesis revisited: Regulators and clinical implications

Roberto Ronca[1] | **Mohammed Benkheil**[2] | **Stefania Mitola**[1] | **Sofie Struyf**[3] | **Sandra Liekens**[2]

[1]Experimental Oncology and Immunology, Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy

[2]Laboratory of Virology and Chemotherapy, Rega Institute for Medical Research, Leuven, Belgium

[3]Laboratory of Molecular Immunology, Rega Institute for Medical Research, Leuven, Belgium

Correspondence
Sandra Liekens, Laboratory of Virology and Chemotherapy, Department of Microbiology and Immunology, Rega Institute for Medical Research, University of Leuven, Herestraat 49, Postbus 1043, B-3000 Leuven, Belgium.
E-mail: sandra.liekens@kuleuven.be

Abstract

Since Judah Folkman hypothesized in 1971 that angiogenesis is required for solid tumor growth, numerous studies have been conducted to unravel the angiogenesis process, analyze its role in primary tumor growth, metastasis and angiogenic diseases, and to develop inhibitors of proangiogenic factors. These studies have led in 2004 to the approval of the first antiangiogenic agent (bevacizumab, a humanized antibody targeting vascular endothelial growth factor) for the treatment of patients with metastatic colorectal cancer. This approval launched great expectations for the use of antiangiogenic therapy for malignant diseases. However, these expectations have not been met and, as knowledge of blood vessel formation accumulates, many of the original paradigms no longer hold. Therefore, the regulators and clinical implications of angiogenesis need to be revisited. In this review, we discuss recently identified angiogenesis mediators and pathways, new concepts that have emerged over the past 10 years, tumor resistance and toxicity associated with the use of currently available antiangiogenic treatment and potentially new targets and/or approaches for malignant and nonmalignant neovascular diseases.

ABBREVIATIONS: Ang, angiopoietin; BM, basement membrane; BMP, bone morphogenetic protein; CAM, chorioallantoic membrane; CPT, carnitine palmitoyltransferase; CRC, colorectal cancer; CSC, cancer stem cell; Dll4, delta-like ligand 4; EC, endothelial cell; ECM, extracellular matrix; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; EPC, endothelial progenitor cell; Eph, ephrin receptor; ATS: FA, fatty acid; FGF2, basic fibroblast growth factor; FOXO1, Forkhead box protein O1; GBM, glioblastoma multiforme; GLUT, glucose transporter; HGF, hepatocyte growth factor; HIF, hypoxia-inducible factor; IMG, intussusceptive microvascular growth; mAb, monoclonal antibody; MAPK, mitogen-activated protein kinase; mCRC, metastatic colorectal cancer; MMP, matrix metalloproteinase; MVD, microvessel density; Nrp, neuropilin; NSCLC, non-small cell lung cancer; PDGF, platelet-derived growth factor; PHD, prolyl hydroxylase domain; PFKFB3, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3; PlGF, placenta growth factor; Robo, roundabout; RTK, receptor tyrosine kinase; SDF-1, stromal cell derived factor-1; Sema, semaphorin; SMA, smooth muscle cell actin; TAM, tumor-associated macrophage; TAN, tumor-associated neutrophil; TGF, transforming growth factor; TKI, tyrosine kinase inhibitor; VDA, vascular disrupting agent; VE-cadherin, vascular endothelial cadherin; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; VHL, von Hippel–Lindau; VM, vasculogenic mimicry

KEYWORDS
angiogenesis, growth factors, novel concepts, tumor resistance, endothelial cell metabolism

1 | INTRODUCTION

In 2001, we published a review about the regulators and clinical applications of angiogenesis.[1] At that time (i) angiogenesis and vasculogenesis were considered to have distinct functions, the latter restricted to embryogenesis; (ii) the role of various growth factors, cytokines, proteases, and extracellular matrix (ECM) molecules in the angiogenesis process was acknowledged, but mechanistic and inhibitor studies largely focused on vascular endothelial growth factor (VEGF); (iii) the targeting of genetically stable endothelial cells (ECs) as opposed to genetically unstable and heterogeneous cancer cells was thought to prevent drug resistance; and (iv) promising mouse xenograft studies raised high hopes for the anticancer potential of antiangiogenic drugs. Since then, the knowledge about (pathological) angiogenesis has increased tremendously: novel insights into tumor vascularization have revealed additional ways to increase intratumoral blood flow.[2] Several new classes of angiogenesis regulators, including proteins involved in axon guidance[3,4] and bone formation[5] and microRNAs,[6] were found to regulate various aspects of EC biology. EC metabolism, which only recently received attention, has been put forward as the key determinant of angiogenesis regulation.[7] Antiangiogenic agents failed to provide a consistent and lasting antitumor activity in the clinical setting and were even shown to select for more aggressive tumor cell clones.[8] Vessel normalization instead of vessel regression was found to induce a better antitumor response and to improve the delivery and/or activity of radio- and chemotherapy.[9,10]

The aim of this review is to revisit the original paradigms and to highlight insights and concepts in the angiogenesis field that have emerged over the past years. Rather than presenting an exhaustive study of one particular topic, we prefer to give an overview of several recent discoveries in the field, in order to offer an update of current knowledge and thinking about (pathological) blood vessel growth. Accordingly, we mainly refer to papers that have been published after 2000, but occasionally also to older papers that provided seminal contributions to the field. For an in-depth analysis regarding specific concepts we refer to specialized reviews throughout the text.

In particular, this overview focuses on different modes of tumor vascularization (Part 2) and novel angiogenesis regulators and concepts that have been characterized (Part 3). Each type of blood vessel growth displays specific characteristics and molecular regulators, which may underlie treatment failure using VEGF or VEGF receptor (VEGFR) targeting agents. However, the discovery of novel regulatory mechanisms also provides translational promise. The challenges of antiangiogenic therapy, as well as potential ways to translate novel insights into improved therapy will be discussed (Part 4).

2 | MECHANISMS THAT CONTRIBUTE TO INCREASED TUMOR BLOOD SUPPLY

Already in 1971, Folkman hypothesized that solid tumor growth requires angiogenesis, that is, the formation of new blood vessels from preexisting ones.[11] To date, several mechanisms have been shown to contribute to tumor neovascularization,[2] including sprouting angiogenesis,[12] intussusceptive growth (nonsprouting angiogenesis, characterized by the division of vessels by transluminal pillar formation),[13,14] vascular co-option (hijacking of host capillaries by the tumor),[15] postnatal vasculogenesis (endothelial progenitor cells (EPCs) recruited to the tumor site),[16,17] and vasculogenic mimicry (VM) (blood vessels lined by tumor cells that mimic ECs, Fig. 1).[18]

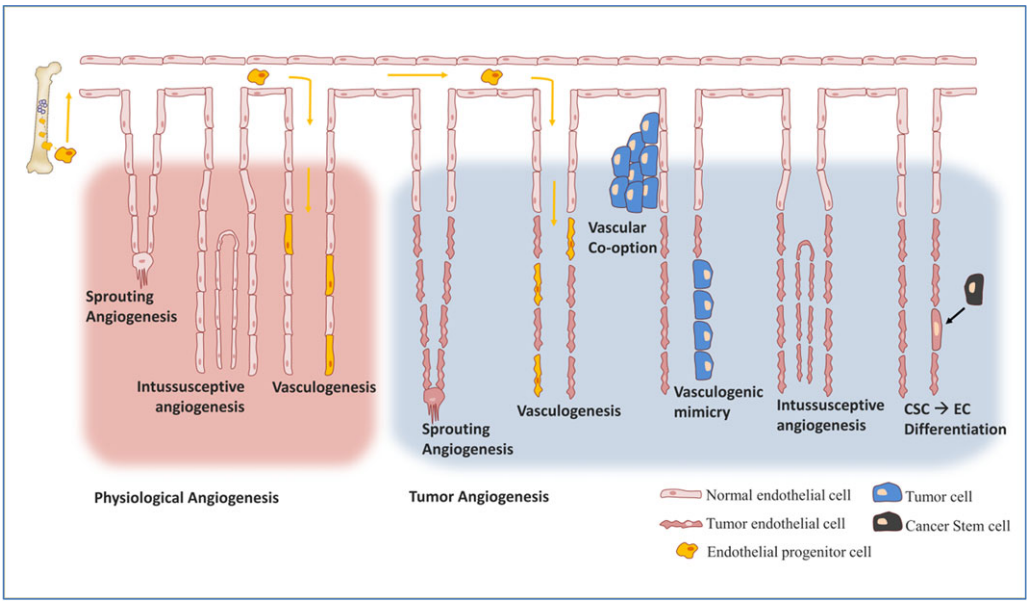

FIGURE 1 Physiological and tumor neovascularization. Physiological neovascularization can occur by sprouting angiogenesis, the recruitment of bone-marrow-derived EPCs that differentiate into ECs (vasculogenesis), or by vessel splitting/intussusception. Tumors can also co-opt preexisting vessels and tumor vessels can be lined by tumor cells instead of ECs (vasculogenic mimicry) or by ECs derived from cancer stem cells (CSCs).

2.1 | Sprouting angiogenesis

Sprouting angiogenesis was originally defined as a multistep process in which activated ECs (e.g., by proangiogenic factors, such as VEGF) migrate and proliferate to form a new capillary vessel.[1,11] This process has meanwhile been further dissected, revealing a tight coordination between migratory tip cells and proliferating stalk cells, and the involvement of several recently identified regulators (see also chapter 3).

According to the revised model of sprouting angiogenesis,[12] activated ECs induce the remodeling of the cell–cell junctions and the basement membrane (BM), along with detachment of pericytes (i.e., perivascular support cells). This allows ECs at the leading edge of the sprout to form filopodia and migrate through the ECM in the direction of an angiogenic stimulus. These migrating tip cells are followed by stalk cells, which proliferate to elongate the new sprout, form a lumen, and recruit pericytes for stabilization. Tip cells of two migrating EC fronts connect and fuse (anastomose) to form a perfused vessel. Upon perfusion, ECs become quiescent phalanx cells, which deposit BM and are covered by mature pericytes.

2.1.1 | Specification of tip and stalk cells

VEGFR2 is expressed on vascular ECs, where it stimulates EC migration, proliferation, survival, and vascular permeability (reviewed in[19]). However, recent data obtained by several research groups while studying sprouting in the mouse retina revealed that not all ECs activated by VEGF respond by directed migration and proliferation. In fact, a specification between tip and stalk cells exists that is controlled by a crosstalk between VEGF and Notch, a crucial player in cell–cell communication (reviewed in[20]).

New sprouts migrate along a chemotactic gradient of VEGF that is produced by the tumor. Consequently VEGF levels are highest at the leading front of the sprout, where activation of VEGFR2 induces the formation of a migrating tip cell. In addition, VEGFR2 signaling causes the expression of the Notch ligand delta-like ligand 4 (Dll4). Dll4-mediated activation of Notch-1 in adjacent ECs suppresses VEGFR2 expression[21–23] and increases VEGFR1 (a VEGF trap) levels.[24–26] This renders the cells less responsive to VEGF and promotes the proliferating stalk cell phenotype.

Thus, an activated tip cell prevents its immediate neighbors from adopting the same phenotype. Moreover, at the leading edge, ECs compete for the tip cell position. Cells with higher Dll4 levels and less Notch signaling will be selected as tip cells.[27]

Notch signaling also inhibits VEGFR3 expression, a receptor of VEGF-C. VEGFR3 is highly expressed in endothelial tip cells in the retina and intersegmental vessels of the zebrafish,[28–30] but is downregulated by Notch signaling in the stalk cells. VEGF-C is less potent than VEGF in stimulating tip cell activity but may become more important when VEGF activity is inhibited. Moreover, VEGF-C/VEGFR3 signaling is required for the stabilization of fusing vascular sprouts.[30]

Surprisingly, and in contrast to earlier findings, Benedito et al.[31] recently demonstrated that Dll4 protein expression in retinal tip cells is only weakly modulated by VEGFR2 signaling and is even maintained in the absence of VEGFR2. Moreover, in settings with low Notch activity, Notch was found to upregulate VEGFR3 resulting in strong, ligand-independent angiogenesis, even in the absence of VEGF/VEGFR2 signaling.[32] These context-dependent effects of Notch may contribute to excessive, nonproductive (poorly perfused and hypoxic) angiogenesis that is seen in tumors treated with Notch inhibitors.[33] Clearly, the status of Notch or VEGFR3 activation in the vasculature might be relevant for patients who do not respond to anti-VEGF treatment.

To identify additional regulators of tip and stalk cell fate, the expression profile of tip and stalk cells has been compared.[34,35] A number of genes are enriched in tip cells, including VEGFR2, platelet-derived growth factor (PDGF)-B, Dll4, matrix metalloproteinase (MMP)14, and the chemokine receptor CXCR4.[35] However, to date, no single protein has been identified that can serve as molecular marker for tip cells. Moreover, tip and stalk cells demonstrate different types of metabolism (see chapter 3). For their migratory behavior, tip cells require large amounts of ATP, which is generated primarily by glycolysis. Conversely, stalk cells divide to elongate the newly formed sprout and consequently require building blocks for rapid macromolecular synthesis (reviewed in[36], see further). Thus, the therapeutic implications of the classification of sprouting ECs into tip and stalk cells stretch beyond the level of angiogenic factors and their receptors.

2.1.2 | Tip cell guidance and vessel anastomosis

Guidance cues are required to direct tip cell migration. This is controlled by several neuronal guidance ligand–receptor interactions (reviewed in[37]). Neuropilins (Nrp) are nontyrosine kinase receptors that bind to class 3 semaphorins (Sema) and to VEGF. Nrp promote tip cell function by enhancing signaling through VEGFR2 and VEGFR3.[38–40] Other important regulators of tip cell guidance are SLIT proteins, which are ligands of the roundabout receptors (Robo), and Ephrins, which activate Eph tyrosine kinase receptors. In particular, ephrin-B2 increases the formation of tip cells and filopodia by regulating VEGFR2/VEGFR3 internalization and trafficking.[41,42] More information regarding the function of these recently discovered angiogenesis regulators is provided in chapter 3.

When tip cells of two growing sprouts meet, they have to be connected. This process, called anastomosis, requires the establishment of new cell–cell junctions. Vascular endothelial (VE)-cadherin is a calcium-dependent adhesion molecule that controls vascular integrity both in developing and existing vessels.[43] However, VE-cadherin is not only present at endothelial cell–cell contacts but also at the filopodia of the tip cells, suggesting that it is involved in vessel anastomosis.[44] Anastomosis is further facilitated by proangiogenic macrophages, which act as bridging cells between anastomosing tip cells, and secrete angiogenic growth factors and proteolytic enzymes.[45–47] Notch activation in the macrophages is required for their recruitment to vascular branchpoints and their interaction with endothelial tip cells.[48]

2.1.3 | Stalk cell proliferation

As mentioned in Section 2.1.1, Notch activation in stalk cells leads to proliferation arrest.[49] However, proliferation of stalk cells is required to elongate the sprout. This is achieved by the expression of Nrarp (Notch-regulated Ankyrin repeat protein), a negative regulator of Notch signaling that destabilizes the Notch intracellular domain.[50] Expression of Nrarp in stalk cells limits Notch signaling at branch points while promoting Wnt signaling. Wnt, in turn, promotes EC proliferation and vessel stability during sprouting. Interestingly, loss of Nrarp was shown to cause vessel

regression. Thus, the balance between Notch and Wnt signaling seems to determine whether new vessels are constructed or destroyed.[51]

2.1.4 | Vessel maturation

Finally, a functional vascular network requires maturation at the level of the vessel wall but also at the network level. In the maturation step, ECs stop migrating and proliferating and the barrier function of vessels is restored. Pericytes play an important role in the stabilization of the nascent blood vessels. They are recruited by PDGF-B, transforming growth factor (TGF)-β1, and angiopoietin (Ang)-1. Reduced pericyte coverage leads to leaky, unstable vessels and is associated with metastasis in cancer patients.[52] Vessel maturation is further improved by the deposition of BM around the quiescent ECs, called phalanx cells.

Importantly, in tumors, angiogenic factors remain overexpressed and vessel maturation does not occur, resulting in the typical abnormal and leaky tumor vasculature (reviewed in[53]). It should also be noted that most insights into tip and stalk cell phenotype and guidance cues have been obtained in developmental rather than tumor models. To explore the full translational potential of these molecular findings, additional studies are required to define the role of these proteins in the cancer setting and, in particular, in metastasis formation.

2.2 | Intussusceptive microvascular growth (IMG)

Intussusceptive growth or intussusception (i.e., growth within itself) is also known as nonsprouting or splitting angiogenesis. This concept was first reported in 1986 by Caduff et al.[54] in the rapidly expanding postnatal lung vasculature of rats. In this type of vessel formation, the capillary wall extends into the lumen to split a single vessel in two. Using electron and confocal microscopy, Paku et al. proposed a mechanistic model for pillar formation, which involves three consecutive steps. First, intraluminal endothelial bridges are formed. Next, the BM is locally degraded and a bridging EC attaches to a collagen bundle in the underlying connective tissue. This collagen bundle is transported into the vessel lumen by pulling forces exerted by the actin cytoskeleton. Finally, maturation of the pillar occurs by invading pericytes and myofibroblasts, which deposit new connective tissue.[55]

Interestingly, this type of blood vessel formation requires only minimal EC proliferation or BM degradation and is therefore less metabolically demanding than sprouting angiogenesis. Moreover, vessels are generated more rapidly by IMG than via sprouting. As such, IMG may have developed to provide a faster response to a tissue's oxygen demand.

IMG has been reported in various tumor types, including colon and mammary carcinoma, melanoma, glioma, and B-cell non-Hodgkin's lymphoma.[56–59] Sprouting angiogenesis and IMG were even detected simultaneously in a single mammary tumor nodule.[60] Moreover, a switch from sprouting to intussusceptive angiogenesis has been observed in relapsing tumors after irradiation or treatment with antiangiogenic agents (see chapter 4). Since capillaries formed by IMG are less leaky and more stable than the typical abnormal tumor vasculature, IMG could be seen as a mechanism contributing to vessel normalization.[13,61] Consequently, rapid vascular remodeling induced by IMG could potentially improve drug delivery and radiation efficacy.

Several proteins are suggested to contribute to IMG, including VEGF,[62] PDGF,[63] basic fibroblast growth factor (FGF2),[63,64] Ang-1,[65,66] and Erythropoietin.[59,67]

In the chicken chorioallantoic membrane (CAM), VEGF expression was found to be high during sprouting angiogenesis, but reduced during intussusception,[13,68] suggesting that high concentrations of VEGF mainly induce sprouting, whereas at lower concentrations, a switch to IMG may occur. These data, although obtained in a developmental model, could explain resistance of relapsed tumors after anti-VEGF therapy.

2.3 | Vascular co-option

Vascular co-option (reviewed in[15]) is the mechanism by which tumor cells surround and hijack host vessels resulting in the incorporation of host–tissue capillaries by the tumor, thereby eliminating the need for new vessel formation. As such, tumors that predominantly get their blood supply through vascular co-option are also called nonangiogenic

tumors. Since the "hijacked" vessels are normal host vessels, they lack the typical abnormal features of tumor vasculature, thus providing a better intratumoral blood flow. Vessel co-option occurs mainly in highly vascularized tissues, such as liver, lungs, and brain. In primary and metastatic lung cancer and liver metastases even 10–30% of tumors were reported to use this alternative blood supply. Consequently, nonangiogenic tumors that appear in these organs are less likely to respond to antiangiogenic therapy.

Vessel co-option was first extensively studied by Holash et al.[69] after implantation of glioma and mammary adenocarcinoma cells in the rat brain, and in experimental lung metastases of Lewis lung carcinoma cells. Although small tumors were considered to be largely avascular, these authors showed that a subset of tumors rapidly co-opts existing host vessels to form an initially well-vascularized tumor mass. However, rapid regression of these co-opted vessels by increased Ang-2 expression led to an avascular tumor and massive tumor cell death. Ultimately, the tumor was rescued by VEGF, which in concert with Ang-2 induced robust angiogenesis at the tumor periphery.[70] Following studies showed that VEGF-overexpressing human melanoma cells induced rapidly growing brain metastases by dilation of co-opted, preexisting vessels.[71]

Vessel co-option has also been demonstrated in non-small cell lung cancer (NSCLC) and lung metastases, derived from breast, colorectal, and renal cancer cases. [72–75] A prerequisite for nonangiogenic tumor growth in the lungs appears to be the ability of the tumor to preserve the parenchymal structures of the lung, which can easily be distinguished on tissue sections. [73] In liver metastases of colorectal carcinomas (CRC), tumor cells were found to replace hepatocytes, preserving the liver architecture and co-opting the sinusoidal blood vessels.[76] This replacement pattern was even more prevalent in breast carcinoma liver metastases, both at the tumor edge and center, and was not accompanied by induction of hypoxia or vascular leakage.[77]

The above-mentioned studies indicate that, at least in well-perfused organs, tumors may grow without the need for new blood vessel formation. However, research conducted on this type of alternative blood supply has been limited, mainly because of difficulties to identify co-opted vessels in the tumor vasculature and to distinguish them from vessels that have been formed through angiogenesis. Most studies use CD31, CD34, or vWF staining to quantify angiogenesis. However, these markers also stain co-opted vessels. Smooth muscle cell actin (SMA), which stains pericytes that cover mature vessels, may better distinguish between co-opted and angiogenic vessels since the latter are less mature and often lack pericyte coverage.[78] Double immunostaining using an EC marker and an antibody against Ki67 or proliferating cell nuclear antigen (PCNA) that detects proliferating cells may also aid in the detection of ongoing angiogenesis. Alternatively, phase contrast magnetic resonance imaging has been shown to detect vascular remodeling in co-opting brain.[79]

In conclusion, additional studies are warranted to understand the mechanism of vascular co-option and to investigate its impact on antiangiogenic tumor therapy and recurrence of liver, lung, or brain metastases. In particular, since co-opted vessels are structurally diverse from angiogenic vessels they are likely to be differentially regulated. It may therefore be expected that these vessels do not respond to the factors that drive angiogenic blood vessel growth. The latter is also reflected by increased co-option occurring in tumors resistant to antiangiogenic therapy (see chapter 4).

2.4 | Role of EPCs in tumor neovascularization

Accumulating data indicate that EPCs are not only involved in embryonic development but also in adult vasculogenesis (reviewed in[16,17,80]). This concept was first demonstrated by Asahara et al. in 1997,[81] who isolated circulating cells with properties of progenitor and ECs from human peripheral blood. These cells (i) differentiated into ECs in vitro and (ii) contributed to angiogenesis in a mouse model of hind limb ischemia, and were therefore considered to be EPCs. A subsequent study by these authors showed that bone marrow EPCs not only have therapeutic potential but are also involved in tumor neovascularization.[82] The role of EPCs and vasculogenesis in cancer has since then been extensively studied.

In physiological conditions, EPCs are maintained in the bone marrow. They reside in a "stem cell niche" exposed to high local concentrations of the chemokine stromal cell-derived factor 1 (SDF-1/CXCL12), which attracts and binds

CXCR4-expressing EPCs.[83] Also, integrins are involved in the retention of EPCs in the bone marrow. In particular, integrin $\alpha 4\beta 1$ mediates adhesion of EPCs to fibronectin and vascular cell adhesion molecule (VCAM)-1.[84]

The mobilization of EPCs from the bone marrow to the peripheral blood and the tumor site is regulated by various cytokines and proteolytic enzymes that are released from the tumor microenvironment.[85–87] So far, VEGF/VEGFR and CXCL12/CXCR4 are considered the key pathways regulating bone marrow-EPC mobilization.[85,86] The EPC-mobilizing activity of both cytokines is dependent on MMP9, whose expression is induced by VEGF. In the initial step of EPC mobilization MMP9 induces the release of EPCs into the circulation.[85] VEGF-induced mobilization of EPCs also involves downregulation of $\alpha 4\beta 1$ integrin in the bone marrow.[88]

EPCs migrate to sites of vascular damage, where they increase vascularization and improve blood flow. Increasing numbers of circulating EPCs have also been observed in many cancers, including some types of leukemia, lymphoma, and breast cancer.[16] The migration of EPCs to the tumor site is dependent upon chemokine gradients. Once in the tumor bed, EPCs may either differentiate into ECs or stimulate tumor angiogenesis by producing proangiogenic factors.

However, the exact role played by EPCs in tumor angiogenesis remains unclear. In particular, the number of EPCs incorporated into nascent tumor vessels varies between 50% to undetectable. These differences are most likely related to the lack of a consensus definition of EPCs. Indeed, EPCs share many markers with hematopoietic stem cells, both cell linages deriving from a common progenitor, the hemangioblast (for more information see[16]). Moreover, the definition of EPC is hampered by the fact that there are two types of cells: proangiogenic hematopoietic cells (early EPCs) and outgrowth ECs (late EPCs). Also, EPCs from different origins (bone marrow vs. peripheral blood) express different markers. As long as the exact definition, origin, and characteristics of EPCs are under debate their role in tumorigenesis will remain unclear.

2.5 | Vasculogenic mimicry

VM refers to the plasticity of cancer cells to form vascular channels. Consequently, tumor cells are directly exposed to the blood, thus facilitating tumor cell invasion and dissemination. Increased metastasis may explain the correlation between VM formation in malignant tumor tissue and poor patient clinical outcome.[89] VM was first described in 1999 by Maniotis et al.[90] in aggressive metastatic melanoma and has since then been reported in a variety of tumors, including various carcinomas, sarcomas, glioblastomas, astrocytomas, and melanomas.[89] In melanoma and glioblastoma the intratumoral hypoxic microenvironment was suggested to induce the phenotypic switch to VM.[91,92]

Tumor cells that contribute to VM present a multipotent phenotype. However, unlike embryonic stem cells, these cells lack critical checkpoint regulation, rendering them highly aggressive.[93] Using microarrays it was demonstrated that melanoma cells that line VM channels show characteristics of both malignant and ECs.[94] Recent studies even indicate the involvement of cancer stem cells (CSCs) in VM (see Section 2.6).

Remodeling of the ECM is critical for the formation of VM channels and to connect these tumor cell-lined vessels to the ECs of the host vasculature. In particular, the metalloproteinases MMP2 and MMP14 are crucial for VM formation by promoting the cleavage of the laminin-5γ2-chain into fragments that stimulate migration and invasion.[95] One of the first proteins that was shown to be involved in VM in aggressive melanoma is VE-cadherin. VE-cadherin phosphorylates the epithelial-associated kinase EphA2 (see chapter 3), which increases MMP production. Accordingly, knockdown of VE-cadherin or EphA2 inhibits VM.[96,97]

In vivo data attribute an important role for VEGF in VM formation. In melanoma, VEGF/VEGFR1 and downstream PI3K signaling act in co-operation with integrin-mediated signaling pathways to induce VM.[98] In ovarian cancer, VM is mediated by VEGF-induced expression of EphA1, MMP2, and MMP9.[99]

In conclusion, VM has been reported in many tumor types and its occurrence is associated with poor clinical prognosis. However, the study of VM is hampered by the lack of methods to clearly distinguish VM vessels from normal EC lining. Besides the fact that these tumor-derived ECs still express some tumor-specific markers, they are indistinguishable from ECs. As such, it has been difficult to link antiangiogenic treatment failure to the appearance of VM-type vessels. Recent data, which show the involvement of hypoxic, angiogenic, and stem cell pathways (see Section 2.6) in

VM formation, indicate that VM-containing tumors might be more sensitive to broad-acting drugs compared to specific VEGF-targeting agents. In fact, an in vitro differentiation assay suggested that hypoxia, but not VEGF, is an important factor in the differentiation of glioblastoma multiforme (GBM) tumor cells to ECs.[92]

2.6 | Role of CSCs in tumor neovascularization

CSCs are considered to be major drivers of tumor progression due to their self-renewal capacity and limitless proliferative potential. However, these cells may also promote tumor vascularization by increasing EPC recruitment. A comparison of rat glioma xenografts containing a high and low fraction of CSCs showed that CSC high tumors express increased levels of VEGF and CXCL12, which promote the mobilization and recruitment of EPCs.[100] CSC-high tumors also displayed an increased microvessel density (MVD) and blood perfusion. In vitro, CSC-high cultures induced higher levels of EC proliferation and tube organization compared with CSC-low cultures.

Recent studies even raised the possibility that CSCs may also differentiate to ECs and give rise to true endothelial linings in some human cancers, including lymphoma and glioblastoma.[101,102] This hypothesis was supported by the fact that a subpopulation of ECs within glioblastomas harbors the same somatic mutations as tumor cells, such as amplification of the epidermal growth factor receptor (EGFR).[101] Moreover, a fraction of $CD133^+$ stem cells is multipotent and capable of differentiating into tumor and endothelial lineages. Selective targeting of ECs generated by glioblastoma stem-like cells (GSCs) in mouse xenografts resulted in tumor reduction, indicating the functional relevance of the GSC-derived endothelial vessels.[102]

Moreover, Cheng et al.[103] elegantly demonstrated that CSCs may also differentiate into pericytes. Analysis of human GBM specimens using lineage-specific fluorescent reporters showed that most pericytes are derived from neoplastic cells. These GSCs are recruited to ECs by CXCL12/CXCR4 signaling and differentiate into pericytes by TGF-β. In addition, elimination of GSC-derived pericytes disrupted the vasculature in GBM, resulting in reduced tumor growth.[103,104]

CSCs, as identified by expression of the stem cell markers CD133 and CD44 (VE-cadherin), also directly increase vascularization by VM in aggressive renal cell carcinoma.[105] CD133 expression further correlated with VM in triple-negative breast cancer specimens[106] and the GBM cell line U87.[107] The mechanism by which CSCs induce VM is not yet completely understood, but the VEGF/VEGFR2 and Nodal/Notch pathways seem to be involved.[107,108]

3 | NOVEL ANGIOGENIC REGULATORS AND CONCEPTS

The molecular dissection of angiogenesis mechanisms has led to the discovery of novel types of angiogenesis regulators (Fig. 2), including proteins involved in bone formation and neurovascular guidance. Meanwhile, microRNAs were discovered and found to regulate various aspects of EC biology. Finally, EC metabolism has been put forward as the main engine that drives angiogenic processes. These concepts will be highlighted here.

3.1 | Bone morphogenetic proteins (BMPs)

BMPs belong to the TGF superfamily and consist of about 20 proteins that regulate multiple biological processes in development and morphogenesis.[5] BMPs are synthesized in the cytoplasm as dimeric precursor proprotein complexes, which are cleaved by serine endoproteases before secretion.[109,110]

BMP signaling is mediated by heteromeric combinations of type 1 (ALK1 /SKR3, ALK2/ACTRIA, ALK3/BMPRIA, and ALK6/BMPRIB) and type 2 (ALK4/BMPRII, ALK5/ActRIIA, ALK7/ActRIIB) transmembrane serine/threonine kinase type receptors. Upon BMP interaction, the type 2 receptors transphosphorylate the type 1 receptors leading to the phosphorylation and nuclear translocation of similarity to (the Drosophila gene) Mothers against decapentaplegic (Mad) SMAD proteins. BMP receptors can also initiate SMAD-independent signaling, thus increasing the fine tuning of signals activated by BMPs. Non-SMAD signaling pathways include the phosphoinositide 3-kinase

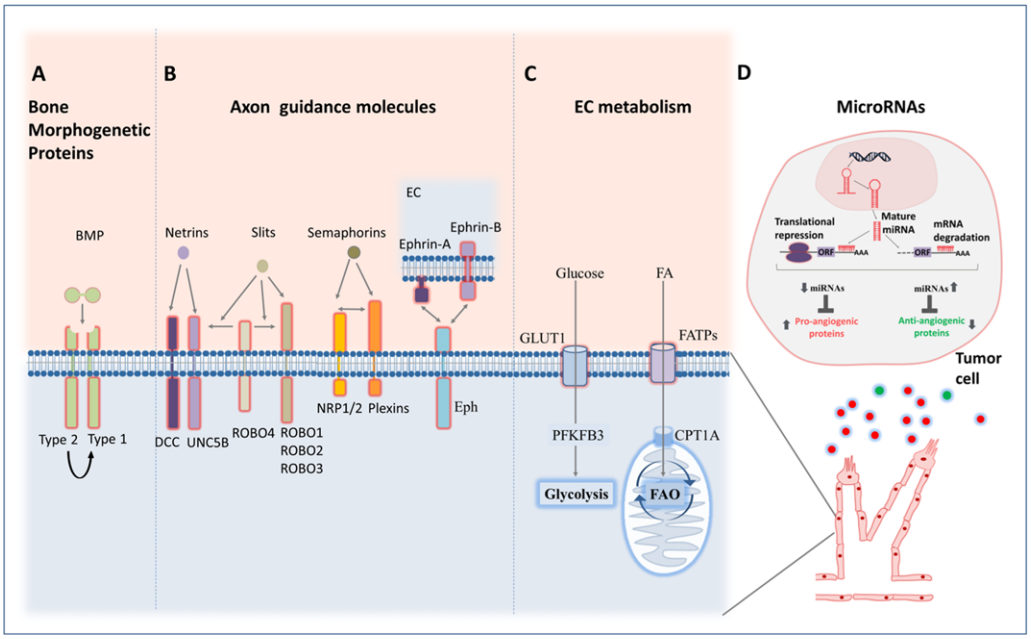

FIGURE 2 Novel angiogenesis regulators. Several novel classes of angiogenesis regulators have recently been identified. (A) BMPs are dimers that bind to heterodimeric combinations of type 1 and type 2 receptors. Upon BMP interaction, type 2 receptors transphosphorylate type 1 receptors, leading to intracellular signal transduction. BMPs include proangiogenic (BMP2/4) and antiangiogenic (BMP9/10) members. (B) Four families of axonal guidance ligand/receptor complexes are implicated in tumor angiogenesis: (1) Netrins, which bind to DCC or UNC5B receptors, (2) Slits-roundabout receptors (Robo), (3) semaphorins, which bind plexins and neurophilins (NRP), and (4) Ephrin-Ephrin receptors (Eph). The extracellular domain of Robo1-3 directly interacts with the Slits, whereas Robo4 requires a coreceptor (e.g., Robo1/2 or UNC5B) for slit binding. Plexins are the main receptors for semaphorins, but a subset of the semaphorins requires the presence of neuropilin coreceptors (Nrp1 and Nrp2). Ephrin-A ligands are attached to the cell membrane by a GPI linker whereas Bephrin-B ligands are transmembrane proteins. Eph receptors as well as class B ligands transduce intracellular signaling. (C) Glycolysis and FA oxidation (FAO) have recently been put forward as major drivers of vessel sprouting. ECs are highly glycolytic and use glucose for energy production. Glucose enters ECs via glucose transporters (GLUTs) and is converted to pyruvate by a cascade of glycolytic enzymes, including PFKFB3 (6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3). FA enters ECs though FATPs and subsequently enters the mitochondria though carnitine palmitoyltransferase 1A (CPT1A). FA-derived carbons enter the TCA cycle and are incorporated in nucleotide precursors for DNA replication. (D) Various microRNAs play a role in vascular remodeling and angiogenesis. MicroRNAs targeting proangiogenic factors are typically downregulated in cancer, whereas angiogenic microRNAs stimulate angiogenesis by reducing angiostatic signals. Angiogenesis is further regulated by tissue-specific and/or hypoxia-induced microRNAs.

(PI3K)-Akt-mTOR pathway, the small GTPases Rho, Rac, and Cdc42, and the Ras-Erk-Mitogen-activated protein kinase (MAPK) pathway.[111,112] BMP signaling may be affected by membrane-associated coreceptors including Bambi, DRAGON, ROR2, Endoglin, and Betaglycan (TbetaRIII).[113–115] The latter two lack kinase activity but regulate vascular development by increasing the binding of BMPs and by modulating BMP receptor trafficking and cell localization.[116,117]

Beyond their importance in embryonic development, BMPs exert proangiogenic roles in vitro and in vivo directly by activating ECs, or indirectly by inducing the expression of other proangiogenic factors. BMP2/4/6/7 stimulate ECs to proliferate, migrate, and to reorganize in tube-like structures via the activation of SMAD1/5, Erk 1/2, and Id1 expression.[118–121] In vivo, recombinant BMP2/4 enhance angiogenesis in the chicken CAM assay and in Matrigel plugs. BMP2/4 further induce a large increase in the size and number of blood vessels in different murine tumor models, acting early in the establishment of a tumor's blood supply. [122,123] In several physiological and pathological conditions

BMPs influence the vasculature in an indirect way by regulating the expression of growth factors and their receptor(s) including VEGF, FGF, VEGFR2, and VEGFR1.[124–126]

BMP9 and BMP10 instead mainly mediate the late phases of angiogenesis, as they suppress EC migration and proliferation.[118] These BMPs are functionally equivalent ligands of the endothelial-specific receptor ALK1. They are supplied via the bloodstream (2 and 12 ng/mL in healthy humans) and are in constant interaction with the apical side of ECs where they control quiescence of adult blood vessels.[127–130] During embryogenesis, two distinct functions of BMP10 have been described. BMP10 supports vascular development via ALK1-dependent signaling in ECs, which can be functionally substituted by BMP9. On the other hand, BMP10 regulates heart development in a BMP10-exclusive manner. In postnatal vascular development both ligands serve a redundant role.[131,132] BMP9 and BMP10 prevent VEGF and FGF2-induced sprouting angiogenesis in vitro and in several in vivo assays, including Matrigel plug assay, CAM assay, and developmental retinal neovascularization in vivo via activation of downstream SMAD 1/5 pathways.[133] BMP9 not only inhibits angiogenesis but also destabilizes already formed vessels.[130] Moreover, the BMP9/ALK1 pathway inhibits neovessel formation in mouse models of age-related macular degeneration.[134]

Recently, particular attention was reserved to BMP9/10-ALK1 signaling as an alternative target for the development of antiangiogenic therapies (reviewed in[135,136]). ALK1 is widely expressed on prostate, skin, thyroid, kidney, ovary, lung, pancreas, and liver tumor blood vessels. Anti-ALK1 can decrease tumor growth and angiogenesis when combined with a VEGFR inhibitor in a human/mouse chimera tumor model.[137] Two main pharmacological inhibitors, an ALK1-Fc fusion protein (Dalantercept/ACE-041),[138–140] which serves as BMP9 (and BMP10) ligand trap, and a fully human antibody against the extracellular domain of ALK1 (PF-03446962)[141–143] are currently under clinical development for cancer treatment.

3.2 | The neurovascular link: Axon guidance molecules

While studying the angiogenic process at a molecular level, a strong resemblance was noted between vessel sprouting by tip cells and neural development/axon guidance. Four canonical families of axon guidance cues implicated in tumor angiogenesis are distinguished: netrins, which binds distinct receptor types (deleted in colorectal cancer (DCC) or UNC5 homologs); semaphorins, which bind plexins and neuropilins; Ephrins-Ephrin receptors (Eph); and Slits-roundabout receptors (Robos).[3,4] Here we focus on their function in (tumor) angiogenesis.

Although the netrins were the first axon guidance molecules to be identified, the role of netrin 1 and netrin 4 in angiogenesis has yet to be understood since contradictory results have been obtained in vitro and in vivo.[144,145] Besides, expression of netrin 1, the most studied netrin, has only been demonstrated in few tumors, although its receptor UNC5B is strongly expressed in tumor blood vessels.[144]

3.2.1 | Slits and robo receptors

Slits are secreted ECM proteins that share a high degree of structural conservation. In mice and humans three Slits have been identified (Slit1, Slit2, and Slit3).[146] In order to gain axon guidance function Slits need to be proteolytically cleaved to release the active Robo-binding N-terminus.[147] The effect of SLIT processing on endothelial receptor activation is not yet clear.[148]

The Robo family of receptors is highly conserved through evolution. In mammals four Robo receptors are present: Robo1/Dutt1, Robo2, Robo3/rig1, and Robo4/magic roundabout.[146] While Robo1-3 contain a high degree of structural and functional similarity, the extra- and intracellular domain of Robo4 is smaller and Robo4 acts distinctly.[3] Robo receptors belong to the immunoglobulin (Ig) superfamily of cell adhesion molecules. The extracellular domain of Robo1-3 interacts with the Slits, whereas Robo4 requires a coreceptor as it does not directly bind Slit ligands. This coreceptor might be a heparan sulfate proteoglycan (e.g., Syndecan), Robo1, Robo2, or UNC5B (the chemorepellent receptor of Netrin-1).[149–152] Proteoglycans also stabilize interactions between Slits and Robo1-3 and heparan sulfate glycosaminoglycans concentrate and localize Slits in the tissue.[153,154] Robo1 and Robo4 are expressed in ECs and regulate both physiological and pathological angiogenesis.[155]

Robo4$^{-/-}$ mice display normal vessel patterning.[152,156] However, during pregnancy, Slit/Robo4 signaling is involved in regulation of the vascular network in the mammary gland when angiogenesis is stimulated by VEGF.[155] The proangiogenic action of VEGF is in this process balanced by Slit/Robo4 interaction, through inhibition of lamellipodia formation.[157] Robo4 maintains the barrier function of mature vessels by inhibiting angiogenic factor-induced endothelial hyperpermeability.[156] Consequently, high expression of Robo4 correlates with a favorable prognosis in early-stage lung cancer.[158]

Slit2 signaling through Robo1 and Robo2 mediates pathological retinal neovacularisation.[159] Blockade of Robo1 by a neutralizing antibody reduces MVD and tumor growth of xenografted melanoma cells[160] and chemical-induced squamous cell carcinogenesis.[161] Whereas normal and hyperplastic buccal mucosa express Slit2 minimally, its expression is drastically upregulated in neoplastic mucosa where it correlates with increased tumor angiogenesis.[161] Slit2 mRNA is expressed in multiple human cancer cell lines and in cancer tissue.[160] The promoter region of Slit2 is frequently hypermethylated in lung and breast cancer reducing Slit2 expression and suggesting a tumor-suppressive role for Slit2.[162] Thus, Slit2 affects both endothelial and tumor cells and may elicit endothelial stimulatory as well as inhibitory actions. This attracting versus repellent action is typical for members of the axon guidance family. In the case of Slits it is at least partly caused by differential receptor activation. Robo1, which directly interacts with Slits, promotes endothelial motility.[150] In contrast, Robo4 that needs a coreceptor for Slit-induced signaling induces endothelial repellence and, in counteraction of VEGF, provides vascular stabilization.[156,163] In addition to differential expression or activation of Robo1/2 versus Robo4 on ECs, other explanations have been proposed for the angiogenic versus angiostatic effect of Slits[148]: (1) the need for a cofactor enforcing the angiostatic effect,[147,164,165] (2) agonistic versus antagonistic effects of the N-terminal fragment of Slit versus full-length Slit, and (3) a cell-dependent processing of Slit.[147,164] Additional research on Slit2/Robo signaling in ECs will hopefully provide a unified concept.[148]

Thus, based on their altered expression in a wide variety of cancer types and their regulatory function on vascular networks, Slits and Robos might serve as targets for cancer treatment. However, their bifunctionality, that is, opposite functions depending on the cellular circumstances, represents a major challenge. In addition, careful drug targeting to the tumor will be needed, because interruption of normal Slit/Robo signaling in nearby tissues could have deleterious effects.[146]

3.2.2 | Plexins, neuropilins, and semaphorins

Like Slit proteins, semaphorins are multifaceted regulatory signals involved in physiological and pathological angiogenesis. Their receptors are expressed on tumor cells, monocyte/macrophages, and ECs in the tumor stroma. As regulators of tumor angiogenesis, tumor growth, cancer cell invasiveness, and metastatic spreading, they are potential therapeutic targets in cancer.

In vertebrates, the semaphorin family contains around 20 genes that are classified based on common structural features of the encoded proteins.[4,166] All semaphorins contain a typical sema domain, harboring sites for semaphorin dimerization and receptor binding, which is located close to the N-terminus. This domain is also present in the semaphorin receptors of the plexin family. Class 3 semaphorins are secreted, whereas other semaphorins are membrane-anchored or transmembrane proteins that can be further processed into soluble forms by specific proteases. The active forms of several class 3 and class 6 semaphorins are homodimers.[167–169]

Plexins are the main receptors for semaphorins, but a subset of the class 3 semaphorins requires the presence of obligate coreceptor molecules, called neuropilins (Nrp1 and Nrp2).[4] The latter are also coreceptors for VEGFs. Plexins are devoid of TK activity, but are able to activate RTKs (including VEGFR2, FGFR2, and EGFR) in "trans."[170] Multiple semaphorins have been associated with modulation of the tumor vasculature (recently reviewed by[166], including Sema3A, Sema3E, Sema3F, and Sema3G (as inhibitory signals) and Sema4D and Sema6D (as proangiogenic factors)).

Sema3A is an inhibitor of developmental angiogenesis.[171,172] Downregulation of its expression in many types of solid tumors annihilates its negative impact on angiogenesis.[173–175] Also angiostatic Sema3F is downregulated

during tumor progression.[175–177] Systemic and tumor-targeted delivery of Sema3A stabilizes the tumor vasculature and inhibits tumor angiogenesis, metastasis, and tumor growth in multiple mouse models, supporting further investigation of Sema3A-based anticancer therapy.[178]

Some semaphorins seem to control the recruitment and activation of leukocytes. Soluble Sema4A and Sema7A chemoattract and activate monocytes/macrophages inducing the release of proinflammatory and proangiogenic molecules (e.g., CXCL8 or VEGF).[179–181]

Usually, semaphorin signaling pathways lead to inhibition of migration,[4] however, Sema4D/CD100 and Sema6D are angiogenic. Sema4D is a membrane-bound protein expressed in tumor cells, tumor-associated macrophages (TAMs),[182] and platelets, but the extracellular domain can be cleaved and released from producer cells by MMPs. Soluble Sema4D retains the biological activity of membrane-bound Sema4D. Binding of Sema4D to plexin-B1 stimulates angiogenesis directly through activation of plexin-B1 and the downstream signaling pathways PI3K/Akt, Rho, and NF-kB, and indirectly via transactivation of the hepatocyte growth factor (HGF) receptor Met.[183] Angiogenic Sema6D signals through Plexin-A1, which forms complexes with, and activates, VEGFR2.[184]

3.2.3 | Ephrins and ephrin receptors (Eph)

The Eph (erythropoietin-producing hepatocellular carcinoma) receptors, which are TKs, and their membrane-anchored ephrin (Eph receptor interacting protein) ligands form two large families.[185,186] Ephrin-A ligands are attached to the cell membrane via a glycosylphosphatidyl-inositol (GPI) linker, whereas ephrin-B ligands are transmembrane proteins. The receptor–ligand interactions are highly promiscuous.[187] Remarkably, both the receptors and the Class B ligands transduce intracellular signals. Classical forward signaling (i.e., RTK signaling) is activated by an ephrin through its Eph receptor on a neighboring cell. Reverse signaling occurs when a Class B ephrin mediates (after interaction with its receptor) signaling in the cell on which the ephrin is expressed. In many cases forward signaling leads to repulsion of the Eph-expressing cell. However, a repulsive Eph-ephrin pair can in other circumstances also promote adhesion. Of note, transmembrane semaphorins (Classes 4 and 6) may also transduce reverse signals.[170]

Strong genetic evidence supports an essential role for ephrin-B2 and EphB4 in the development of blood and lymph vessels during embryogenesis.[188–191] In addition, ephrin-B2 assists in stabilization of vessel walls during vascular maturation allowing cell spreading and focal adhesion.[192]

Besides regulating embryonic vessel formation, ephrin-B2 and EphB4 play a role in neovascular eye disorders.[185] Furthermore, several Eph receptors and ephrin molecules are upregulated in tumors and may affect tumor growth and vascularization. For instance, EphA2 and its ligand ephrin-A1 are expressed in breast, ovarian, prostate, lung, and skin cancer.[187,193] In breast tumor cells overexpressing EphA2, EphA2 signals in a ligand-independent fashion through crosstalk with EGFR and HER2, thereby promoting tumor growth.[194] In the tumor environment, expression of EphA2, which is absent on quiescent vasculature, is induced in ECs via interaction with ephrin-A1 present on tumor ECs and tumor cells. Subsequent forward signaling through EphA2 stimulates angiogenesis[195] and vascular permeability.[196] Blocking EphA receptor activation or gene deletion in mice reduces the size and vascularization of experimental tumors.[197] Similarly, high expression of ephrin-B ligands and EphB receptors in some human cancers correlates with poor prognosis.[198,199] Ephrin-B2, which is upregulated in ECs by hypoxia and VEGF, is expressed on the blood vessels of many tumors.[195] Through activation of ephrin-B2 on ECs, tumor cell expressed EphB4 promotes angiogenesis.[200] Several approaches targeting the activity of ephrin-B2 and/or EphB4 reduced the growth and vascular network of tumors.[201,202] However, certain Eph receptors exert tumor-suppressive activity[203] and therefore more research is needed to exactly decipher the signaling pathways and activities of Ephs and ephrins in specific cell or cancer types.

3.3 | miRNAs as master regulators of tumor angiogenesis

MicroRNAs (miRs) are short (~22-nucleotides in length) endogenous noncoding RNAs that were discovered in vertebrates in 2001.[204] They negatively regulate the expression of target genes at a posttranscriptional level, thereby affecting many cellular processes.[205,206] MiRs promote the cleavage of the target mRNA or inhibit its translation through complementary base pairing at the 3′ untranslated region (UTR).[207] Expression of miRs is strictly regulated in

TABLE I Angiogenic factors targeted by miRs

Angiogenic factor	Targeted by	Effect on angiogenesis	Ref.
Ang-1	miR-204	–	208
Ang-2	miR-542-3p	–	209
Dll4	miR-30 family/miR-150	+	210–212
FGF2	miR-15a/miR-16/miR-152/miR-195/miR-205/miR-497/miR-503/miR-646	–	213–220
HIF-1	miR-17-92/miR-107/miR-135b/miR-429/miR-497/miR-519c	–	221–226
MMP14	miR-9/miR-181a-5p/miR-337-3p/miR-584-5p	–	227–231
PTEN	miR-17-92/miR-21/MiR-29a/miR-382/miR-4534/miR-494	+ (induces Akt and Erk pathway)	232–238
TSP-1	miR-17-92/miR-194/miR-200a/miR-487b	+	239–242
VE-cadherin	miR-27a-3p/miR-125b	–/Nonfunctional blood vessels	243,244
VEGF	miR-15a/miR-16/ miR-29b/miR-17-92/miR-101/miR-125a/miR-126, miR-145/miR-184/miR-190/miR-192/miR-195/miR-200b/miR200c/miR-205/miR-497/miR-503/miR-638	–	213,214,220,223,245–259

+, stimulation; –, inhibition; Ang-1/2, angiopoetin-1/2; Dll4, delta-like canonical Notch ligand 4; FGF2, basic fibroblast growth factor; HIF-1, hypoxia-inducible factor-1; MMP14, matrix metalloproteinase-14; PTEN, phosphatase and tensin homolog; TSP-1, thrombospondin-1; VE, vascular-endothelial cadherin; VEGF, vascular endothelial growth factor.

a time- and tissue-specific manner, allowing cell-specific functions. Since 2008, an increasing number of miRs has been identified that regulate the expression of angiogenic factors or interfere with angiogenic signaling pathways (for an overview, see Table I and reviewed in[6]).

MiRs targeting proangiogenic factors are typically downregulated in cancer, whereas angiogenic miRs reduce angiostatic proteins. As such, both can contribute to the angiogenic switch during cancer progression. Interestingly, miR expression may be transcriptionally controlled by (anti)angiogenic proteins, adding further complexity to miR-based angiogenesis regulation.

3.3.1 | Endothelial-specific miRs: miR-126

Some miRs are endowed with tissue-specific actions. A crucial player in EC biology is miR-126, which is specifically enriched in ECs and EPCs. During embryonic vasculogenesis, miR-126 maintains vessel integrity by enhancing the proangiogenic activity of VEGF and FGF, via suppression of negative regulators of the Ras/MAPK and PI3K/Akt pathways.[260,261] However, miR-126 also binds to the 3′UTR of VEGF mRNA, thereby reducing the expression of VEGF.[247] As such, its role in tumor growth is not clear and, depending on the tumor type, it may elicit a tumor-promoting or suppressing function (reviewed in[262]).

miR-126 is downregulated in several cancer types, including lung,[263] breast,[264] colorectal,[265] and gastric cancer[247] by promoter methylation of its host gene, EGF-like domain 7 (Egfl7).[265,266] A correlation was shown between miR-126 downregulation and poor metastasis-free survival of breast cancer patients.[266] Moreover, downregulation of miR-126 was found to correlate with increased MVD and VEGF expression in gastric cancer tissues.[247] Conversely, miR-126 was shown to suppress EC recruitment to metastatic breast cancer cells, metastatic angiogenesis, and colonization through coordinate targeting of novel proangiogenic genes and biomarkers of human metastasis (i.e., insulin-like growth factor binding protein 2, PITPNC1, and c-Mer tyrosine kinase).[267]

3.3.2 | Cell-type and context-dependent miR: miR-17-92

The miR-17-92 cluster was among the first miRs that were linked to tumor angiogenesis. miR-17-92 is a polycistronic miR cluster that contains multiple miRs (miR-17, miR-18a, miR-19a/b, miR-20a, and miR-92a). Each of these miRs holds the potential to regulate several target mRNAs. As such, the functions of this miR cluster are very diverse and cell type and context dependent. miR-17-92, also called oncomir-1, is upregulated by the transcription factor Myc in several cancers.[268,269] Thrombospondin (TSP)-1, one of the main endogenous inhibitors of angiogenesis, is targeted by miR-19.[268,270] Consequently, miR-17-92-transduced tumor cells form larger and better perfused tumors, which correlate with downregulation of TSP-1.[268] Recently, treatment of ECs with VEGF was shown to trigger the expression of all miR-17-92 cluster members by activation of MAPK and the transcription factor Elk-1 (member of the E26 transformation-specific (ETS) oncogene family), which binds to the miR-17-92 cluster promoter sequence. This resulted in repression of PTEN (phosphatase and tensin homolog), a tumor suppressor that inhibits Akt/PI3K signaling. Consequently, VEGF-induced miR-17-92 cluster expression contributes to the angiogenic switch of ECs and was found to be essential for EC proliferation and sprouting.[271]

However, in an in vivo setting, miR-17-92 biology is likely to be more complex, and dependent on the regulation of individual (pro- and antiangiogenic) members of this cluster as well as non-EC-derived miR-17-92 cluster members. Indeed, miR-17-92 also elicits antiangiogenic activity by targeting TGFBR2, VEGF, and the transcription factor hypoxia-inducible factor (HIF)-1α in colorectal cancer.[223] Moreover, whereas miR-17/20 exhibits antiangiogenic activity in ECs, it does not affect tumor angiogenesis, indicating a context-dependent regulation.[272]

3.3.3 | miRs Regulate Cellular Adaptation to Hypoxia

Further experimental evidence suggests that miRs are important components of cellular adaptation to hypoxia. A number of miRs are upregulated by HIF-1α. The predominant hypoxia-inducible miR is miR-210. HIF-1α binds to a hypoxia-response element (HRE) on the proximal miR-210 promoter. MiR-210 target genes can be classified into five major functional categories: mitochondrial metabolism, cell cycle control, angiogenesis, apoptosis, and DNA damage repair. Expression of this hypoxamiR is greatly induced in pancreatic, breast, head and neck, lung, colon, and renal cell lines after exposure to hypoxia.[273] Furthermore, miR-210 expression has been linked to bad prognosis in patients with soft tissue sarcoma, breast, head and neck, and pancreatic cancer (reviewed in[274,275]). miR-210 also emerged as a potential therapeutic target in diffuse large B-cell lymphoma.[276]

Whereas miR-210 is widely expressed, other miRs respond to decreased oxygen tension in a more tissue-specific manner. Moreover, HIF-1α expression itself is regulated by several miRs, such as miR-497 in breast carcinoma,[224] adding another level of complexity to the classic hypoxia-regulated gene network.

3.4 | EC Metabolism

The role of EC metabolism in tumor angiogenesis has been completely overlooked during the past 40 years. Still, recent concepts indicate that angiogenesis is not only regulated by the balance between pro- and antiangiogenic factors, but also by changes in EC metabolism (reviewed in[7]). Indeed, activated ECs rapidly switch from quiescence to angiogenic sprouting and must be able to adjust their metabolism accordingly. Thus, metabolic changes may significantly impact EC behavior and activity.

3.4.1 | Glucose metabolism

Despite their privileged position having direct access to oxygen in the blood, ECs from macro- and microvessels rely primarily on glycolysis (generating around 85% of their ATP content through this pathway) rather than on mitochondrial respiration to generate energy.[277] In particular tip cells need a high rate of glycolysis.

Glucose enters ECs via glucose transporters (GLUTs) and is converted to pyruvate by a cascade of glycolytic enzymes. Proangiogenic signals like VEGF and FGF2 increase the expression of GLUT-1 as well as that of main glycolytic enzymes, such as PFKFB3 (6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3). Moreover, PFKFB3

harbors hypoxia-response elements and is a target of HIF-1α. Accordingly, knockdown of PFKFB3 in ECs reduces vascular sprouting by impairing tip cell migration and stalk cell proliferation, without affecting the expression of angiogenic factors.[277,278]

Glycolytic intermediates may also be shuttled into other metabolic pathways, including the pentose phosphate pathway (PPP) and hexosamine biosynthesis pathway (HBP). The PPP pathway uses glucose-6-phosphate to generate NADPH for redox control and lipid synthesis and ribose-5-phosphate for nucleotide synthesis. Tumor ECs upregulate genes involved in nucleotide synthesis and shunt glucose metabolites to nucleotide pathways to assure sufficiently high nucleotide pools for cell duplication. NADPH reduces oxidized glutathione, a key cellular oxidant, thereby protecting ECs against oxidative stress.[279] The HBP pathway produces *N*-acetylglucosamine for protein *O*- and *N*-glycosylation. Posttranslational glycosylation status determines the activity and structure of several angiogenic proteins, including Notch,[280] VEGFR,[281] and MMPs.[282] Each of these pathways is important for angiogenesis as its genetic or pharmacological inhibition reduces EC viability, migration, and/or angiogenesis.[280,283–285]

In addition, recent findings highlight the inhibition of c-MYC mediated by FOXO1 (Forkhead box protein O1) as a key mechanism that decelerates metabolic activity of ECs and controls quiescence. MYC ablation impairs glycolysis, mitochondrial function, and proliferation of ECs. Conversely, EC-specific overexpression of MYC fuels these processes, and restoration of MYC signaling in FOXO1-overexpressing endothelium normalizes metabolic activity and branching behavior.[286]

3.4.2 | Fatty acid metabolism

Besides glucose, fatty acids (FAs) represent an important fuel source for ECs. FAs enter the cell through different mechanisms, including membrane-associated FA transport proteins (FATPs), and associate with cytosolic FA-binding proteins (FABPs), which chaperone FAs inside the cell.[287] Proangiogenic signals (VEGF and FGF2) upregulate the expression of FATPs and FABPs, which are required for EC proliferation and sprouting.[288] Recently it has been clarified that, unlike in most normal and cancer cell lines, FA oxidation (FAO) contributes minimally (less than 5%) to ATP synthesis in ECs. Instead, FAO provides carbons for de novo nucleotide synthesis via the tricarboxylic acid (TCA) cycle, thereby promoting EC proliferation and sprouting angiogenesis.[289] Carnitine palmitoyltransferases (CPTs), more precisely CPT1, catalyze the transesterification of long-chain FA-CoA and its transport into the intramitochondrial matrix, thus regulating the rate of FAO. CPT1A is the most abundant isoform in ECs. Its deficiency impairs sprouting and proliferation without altering ATP levels or oxygen consumption.[289]

In conclusion, recent data indicate that metabolic reprogramming, a crucial hallmark of cancer that shifts metabolic pathways to enable sustained growth of cancer cells, also applies to tumor ECs. Consequently, blocking the EC engine might prove more effective than blocking individual drivers, such as VEGF. However, it should be noted that energy production depends on nutrient availability. As such, EC metabolism is modulated by the metabolic program of other cell types in the tumor microenvironment. Accordingly, recent data show that hypoxic TAMs compete with tumor ECs for glucose.[290]

4 | ANGIOGENESIS IN PRACTICE: CLINICAL IMPLICATIONS

The clinical usage of antiangiogenic agents targeting VEGF/VEGFR has shown that the anticancer potential of these drugs is limited and associated with unexpected side effects and tumor resistance, or even the occurrence of more aggressive tumor cells and increased metastasis. Accumulating evidence indicates that these adverse effects may be induced by the expression and activation of alternative angiogenic factors and pathways, the appearance of hypoxia-tolerant or vessel-independent tumor cells or a tumor vasculature that arises in the absence of angiogenesis. Moreover, as novel mediators continue to be discovered, it is clear that the multifactorial nature of neovascularization requires tackling the process at different levels. Various resistance mechanisms and potentially improved treatment options and/or schedules will be discussed here.

4.1 | Why antiangiogenic therapy fails to treat cancer

During the past 10 years, angiogenesis inhibiting drugs have been tested (i) in the adjuvant setting after surgical removal of the primary tumor to prevent local relapse or the growth of micrometastases or (ii) in the neoadjuvant setting to downsize nonresectable to potentially resectable tumors. In most settings, antiangiogenic agents such as bevacizumab and aflibercept (which are classical VEGF-traps) only showed significant activity when combined with cytotoxic chemotherapy, while tyrosine kinase inhibitors (TKIs) also worked as single agents.

Despite the increasing number of data and drug candidates, resistance to antiangiogenic therapy remains a challenging issue that is associated with variable success in the clinic and with poor prognosis for cancer patients. Several mechanisms can account for this therapeutic failure.[291]

4.1.1 | Activation of alternative proangiogenic pathways

Besides VEGF, several additional pathways are implicated in tumor growth and cancer-associated angiogenesis. These alternative ways can sustain tumor growth and blood vessel survival even in response to VEGF/VEGFR blockade.

Inhibition of VEGFR2 in a mouse model of pancreatic cancer resulted after an initial response in tumor regrowth, accompanied by increased vascularization and augmented levels of proangiogenic factors like FGF2, Ang-1, and ephrin-A1.[292] Likewise, FGF2, CXCL12, and placenta growth factor (PlGF) were upregulated in progressive glioblastoma growth after treatment with the pan-VEGFR TKI AZD2171.[293] Together with the "alternative" proangiogenic factors mentioned above several other players have been identified in preclinical studies such as EGF, FGF1, Dll4, HGF, CXCL8, and PDGF-C. [294] Accordingly, increased serum levels of FGF2, HGF, PlGF, and CXCL12 have been observed in patients treated with antiangiogenic agents just before tumor progression and acquisition of resistance.[295] These observations reinforce the rationale for simultaneous targeting of multiple angiogenic drivers. However, the majority of TKIs used to treat patients are multitargeting drugs that nevertheless failed to treat different types of tumors.[296,297]

4.1.2 | Recruitment of proangiogenic stromal cells

The tumor microenvironment contains a heterogeneous and complex mixture of stromal cells (fibroblasts, pericytes, endothelial, mesenchymal, and hematopoietic cells) that actively support tumor growth and are associated with resistance to anti-VEGF therapy.[298]

Antiangiogenic as well as vascular disrupting agents (VDAs), which cause acute hypoxia, trigger the recruitment of EPCs and immune cells to the tumor margins. Various preclinical models strongly suggest a role for neutrophils in mediating tumor angiogenesis and refractoriness to anti-VEGF therapy. Tumor-associated neutrophils (TANs) mediate the angiogenic switch in different tumor models, by producing various growth factors (including VEGF) and secreting active MMP9 that releases FGF2 and VEGF from the ECM.[299] Also the clinical evidence supporting this notion is growing. In myxofibrosarcoma patients, elevated numbers of neutrophils positively correlate with tumor MVD. Moreover, intratumoral infiltration of neutrophils is significantly associated with tumor grade in glioma patients.[300] In NSCLC patients treated with chemotherapy plus bevacizumab, a high number of circulating neutrophils and monocytes and a high neutrophil-to-lymphocyte ratio are associated with poor clinical outcome.[301]

In the last decade a tumor-promoting role has also been attributed to TAMs. Despite their potential role in antitumor immunity, high frequencies of TAMs correlate with poor prognosis in most human cancers.[302] TAMs have proangiogenic activity, and macrophage infiltration in tumors is generally associated with high vascular density.[303,304] Indeed, TAMs infiltrating established tumors acquire an "M2-like" phenotype endowed with promotion of tumor growth and angiogenesis, remodeling of tissues, and suppression of antitumor immunity.[304] M2-like TAMs accumulate in hypoxic tumor areas and display strong proangiogenic activity through the expression of various angiogenic growth factors, cytokines, and proteases.[305]

Furthermore, many other stromal subpopulations may be present or recruited at the tumor site during tumor growth or in response to antiangiogenic treatment. In particular, immature myeloid cells or EPCs that produce growth

factors or physically incorporate into tumor blood vessels have been detected in the tumor microenvironment, where they may foster resistance to antiVEGF therapy.[306,307]

4.1.3 | Alternative mechanisms of tumor vascularization

As mentioned in Chapter 2, tumor vascularization may occur through different mechanisms (Fig. 1), each with its specific characteristics and regulators.[308]

IMG occurs in various tumor types, and is increased in relapsing tumors after irradiation or antiangiogenic treatment.[309–315] In hepatocellular carcinoma xenografts treated with the mTOR inhibitor sirolimus, IMG was observed during treatment and early recovery phase, whereas in control animals the capillary plexus mainly extended by sprouting.[311] Similarly, treatment of mammary carcinomas in mice by radiation or the VEGFR inhibitor PTK787 resulted in transient reduction of tumor growth, followed by post-therapy relapse accompanied by IMG.[309] Comparable observations were made in the RIP-Tag2 and Lewis Lung carcinoma models treated with inhibitors of VEGFR signaling and in renal cell carcinoma[312] and melanoma models treated with TKIs.[316] These data suggest that IMG represents a tumor-protective response to cancer therapy to preserve the intratumoral vasculature. Moreover, the mechanism of IMG is very different from sprouting, indicating that other therapeutic strategies will have to be followed.

Vessel co-option mainly occurs in tumors and metastases of highly vascularized tissues (see Section 2.3). Treatment of mice bearing brain metastases of VEGF-overexpressing melanoma cells with the VEGFR2 TKI ZD6474 showed, despite effective blockage of angiogenesis, sustained tumor progression via co-option.[317] In glioblastoma-bearing mice, an anti-VEGF antibody prolonged survival but increased vascular co-option.[318] Also in an orthotopic human hepatocellular carcinoma (HCC) model, tumors resistant to sorafenib (the TKI approved for systemic therapy of HCC) became more invasive, which facilitated the co-option of liver vessels. Interestingly, whereas 24% of the total vessels were provided by co-option in untreated tumors, this number reached up to 75% in sorafenib-resistant tumors, thus providing the first evidence that vessel co-option is responsible for resistance to antiangiogenic therapy.[319] Also in melanoma metastases taken at clinical relapse in patients undergoing adjuvant treatment with bevacizumab, a mature intratumoral network with low angiogenic activity was noted.[320] Vessel co-option is also associated with resistance to sunitinib in preclinical lung metastasis models[75] and with a poor response to bevacizumab in patients with CRC liver metastases.[321] Moreover, combined inhibition of angiogenesis and vessel co-option was found to be more effective than the inhibition of angiogenesis alone.[321]

4.1.4 | Vascular independence of tumor cells

A prerequisite for the anticancer potential of antiangiogenic agents is the vascular dependence of tumors. However, recent data indicate that tumor cells are heterogeneous in their dependence on neighboring tumor-associated vasculature for survival. Some cancer cells are highly vessel dependent, whereas others can survive in more hypoxic regions of tumors, distal from tumor vessels. Moreover, long-term antiangiogenic therapy may result in the outgrowth of subpopulations of less angiogenesis-dependent malignant cells with an increased capacity to survive in nutrient- or oxygen-deprived tumor areas.[322,323]

Several mechanisms may account for the vascular independence of tumor cells. It has been reported that mice bearing *p53*-deficient (*p53*$^{-/-}$) HCT116 human CRC tumors are less responsive to antiangiogenic combination therapy than mice bearing isogenic *p53*$^{+/+}$ tumors.[324] Alternatively, a metabolic switch in cancer cells may occur, as reported in a mouse model of breast cancer treated with the TKIs nintedanib or sunitinib. After an initial regression, tumors became resistant and resumed growth in the absence of tumor angiogenesis. Distal cells located in avascular areas underwent metabolic reprogramming toward a hyperglycolytic state producing lactate, which was utilized by tumor cells in the vicinity of blood vessels for oxidative phosphorylation.[325]

Altogether these observations confirm that, even if antiangiogenic therapy targets genetically stable ECs in the tumor vasculature, genetic alterations or metabolic switches that decrease the vascular dependence of tumor cells can influence the therapeutic response of tumors to this therapy.

4.2 | Hypoxia induces a more aggressive and resistant tumor type

Hypoxia is one of the main features of solid tumors, and correlates with poor prognosis of cancer patients.[326] Reduced oxygenation of the tumor tissue is also acknowledged as a main cause of resistance to chemo- and radiotherapy. Intratumoral hypoxia can confer chemoresistance by (i) affecting drug delivery and cellular uptake through associated acidity, (ii) upregulation of multidrug resistance protein (MDR) expression, or (iii) by the fact that a number of chemotherapeutics require oxygen to exert their cytotoxic activity.[327] Resistance to radiotherapy is mainly caused by reduced generation of reactive oxygen species (ROS) and decreased DNA damage.[328] In general, radio- and chemotherapy preferentially target rapidly proliferating tumor cells. However, the most resistant cells are quiescent, slowly proliferating, stem-like cell fractions residing in the most hypoxic tumor region.[329,330]

The original paradigm of tumor growth stated that tumors cannot survive or grow in conditions of hypoxia, such as induced by antiangiogenic therapy. However, after treatment with VEGF blockers a proportion of hypoxia-tolerant cancer cells survives in poorly oxygenated niches and adapts to antiangiogenesis by increasing various cellular survival processes.[331] Moreover, dysfunctional tumor vascularity and heterogenic blood supply cause oxygen fluctuations with sporadic reoxygenation periods in cancer.[332] Cells reoxygenated after acute hypoxia may undergo rapid p53-dependent apoptosis. Consequently, cells that lack functional p53 are even more prone to further genomic instability, and potentially tumorigenesis, if they experience reoxygenation after acute exposure to hypoxia.[333]

Hypoxia results in the activation of HIFs. These transcription factors are master regulators of O_2 homeostasis that mediate many transcriptional changes in response to low O_2 tension. HIF-1 consists of a constitutively expressed β subunit and an O_2-sensitive α subunit that is rapidly degraded in normoxic conditions. Degradation involves recognition by prolyl hydroxylase domain (PHD) enzymes, binding to the von Hippel–Lindau (VHL) tumor suppressor protein, ubiquitination, and proteasomal degradation.[334] Under hypoxic conditions, PHD activity is attenuated, leading to HIF-1α protein stabilization, dimerization with HIF-1β, and translocation into the nucleus. The binding of the HIF-1α/β heterodimer to hypoxia response elements (HREs) subsequently induces the transcriptional activation of various genes involved in angiogenesis, metastasis, apoptosis, and glycolysis.[335] As an example, in clear cell renal carcinoma loss of function of the VHL gene, which is responsible for ubiquitination and degradation of HIF-1α, leads to the upregulation of HIF responsive genes, such as PDGF and VEGF.[336]

Nevertheless, the HIF pathway can also be activated in a hypoxia-independent manner by epigenetic changes and mutations that lead to a loss of tumor-suppressor functions and/or a gain of oncogene functions, or in response to cytokines and growth factors.[337] It is worth to remember that HIF-1α and nuclear factor kappa B (NFκB) together govern the malignant and metastatic phenotype of cancer cells through the regulation of a plethora of genes involved in cell survival, migration, invasion, metabolism, and neovascularization.[338]

Overall, hypoxia induces an imbalance in the production of pro- and antiangiogenic factors, which leads to enhanced, rapid, and chaotic blood vessel formation. In particular, hypoxia and HIF-1/2α have been shown to be directly involved in all steps of blood vessel formation. Hypoxia induces the recruitment of EPCs from the bone marrow and their differentiation into ECs.[339] HIF-1/2α stimulate EC proliferation and sprouting of preexisting vessels. Also, HIF1/2α support vessel maturation by inducing Ang-1, PDGF, and TGF-β that recruit smooth muscle cells and pericytes.[340]

Intratumoral neovessels are often abnormal, immature, and leaky, and expanding tumors are extremely demanding in terms of nutrients and oxygen. This results in an hypoxic/angiogenic loop generating a tumor tissue that is highly hypoxic and that contains an excessive/dysfunctional vasculature.[341]

4.3 | Side effects associated with antiangiogenic therapies

Since antiangiogenic compounds are thought to specifically target newly formed, rather than existing vessels or other normal cell types, no or only minor toxicity was anticipated. However, the expanding use of drugs targeting the VEGF signaling pathway in cancer unveiled that these antiangiogenic treatments are often associated with a wide spectrum of toxicities.[342,343] Meta-analyses demonstrated a small risk, around 1.5–2.5% of fatal adverse events with both antiangiogenic TKIs and bevacizumab.

Some adverse effects are shared with conventional chemotherapeutic agents while others are unique and not typically observed with cytotoxic drugs. Specific toxicities associated with VEGF axis inhibition using VEGF-ligand or VEGFR inhibitors include cardiovascular effects (hypertension, thromboembolism, left ventricular dysfunction, cerebrovascular effects) as well as noncardiovascular effects (proteinuria, bleeding/hemorrhage, delayed wound healing, gastrointestinal perforation, fatigue, and dysphonia).[343–346] Other rare class effects of VEGF axis inhibition comprise reversible posterior leukoencephalopathy (RPLS), osteonecrosis of the jaw (ONJ), and microangiopathic hemolysis. One study associated bevacizumab with increased risk of death in combination with taxanes or platinum agents but not in combination with other agents.[342]

The antiangiogenic TKIs display additional class-related toxicities, including gastrointestinal events (diarrhea, nausea), thyroid dysfunction, fatigue, stomatitis, myelosuppression, and cutaneous effects.[347,348] Some of these side effects may reflect the promiscuity of kinase inhibitors, which inhibit multiple other receptors in addition to VEGFRs.

Other drugs like thalidomide, lenalidomide, and pomalidomide, which have both immunomodulatory and antiangiogenic activity, are associated with thrombotic complications, especially when combined with glucocorticoids and/or cytotoxic chemotherapy.

4.4 | Ways to improve antitumor efficacy of antiangiogenic therapy

4.4.1 | Specific targeted therapy versus multitarget compounds—monotherapy versus combination therapy

The prototype of specific/targeted therapies is a monoclonal antibody (mAb), which is usually designed against a very specific target to avoid side effects. Besides bevacizumab, which targets VEGF, some mAbs exhibit antiangiogenic functions due to crosstalk between the signaling pathways that involve VEGF and other growth factors. This is the case for anti-EGFR/HER antibodies, such as cetuximab (Erbitux), trastuzumab (Herceptin), and panitumumab (Vectibix).[349] One of the main drawbacks of this targeted approach is that it often leads to drug resistance, for example, bevacizumab monotherapy induces a rapid reboost in metastatic patients. A less selective molecule aflibercept (AVE0005, Zaltrap) functions as a soluble "decoy" VEGF-trap receptor, preventing the binding of VEGF, VEGF-B, and PIGF to the cell surface receptors. Its wider binding spectrum, when compared with bevacizumab, improved progression-free survival, and overall survival in a clinical trial involving metastatic CRC.[350]

Unlike antibodies and ligand trap molecules, TKIs (Fig. 3, Table II) cross the cell membrane and interact directly with the intracellular domain of receptors and/or other signaling molecules. Due to similarities in the kinase domains, most TKIs offer multitarget activity, good response, and sometimes improved survival rates in phase III clinical trials.[351] VEGFR inhibitors, like axitinib, pazopanib, sunitinib, vandetanib, and vatalanib, block also other receptors, such as PDGFR, c-Kit, EGFR, or RET. Moreover, the VEGFR inhibitor sorafenib targets PDGFR, c-Kit, Flt-3, and BRAF, while sunitinib inhibits Flt-3 and CSF-1R, and nintedanib (BIBF1120) blocks VEGFR, PDGFR, and FGFR signaling.[352] Interestingly, in contrast to agents like bevacizumab and aflibercept that show greater activity when combined with chemotherapy, TKIs generally display single agent activity.

Nevertheless, TKIs have also been investigated in combination with chemotherapy, especially in advanced NSCLC, showing clinical benefits in some studies, but failing to prolong overall survival in others.[353,354] In general, given the role of PDGFR, FGFR, and others RTKs in various aspects of tumor biology, especially regarding pericyte and tumor-cell function, it is difficult to assess the contribution of individual targets to the clinical activity of the multitarget

TABLE II Specific and Multitarget Antiangiogenic Agents

Angiogenic system	Molecule	Commercial name	Type of inhibitor	Target	Ref.
VEGF/VEGFR	Ziv-aflibercept	Zaltrap	Ligand trap	VEGF, VEGF-B, PlGF	355
specific	Pegaptanib	Macugen	Aptamer	VEGF	356
	Bevacizumab	Avastin	Antibody	VEGF	357
	Ranibizumab	Lucentis		VEGF	358
	r84			VEGF	359
	Ramucirumab	Cyramza		VEGFR2	360
	Icrucumab	IMC-18F1		VEGFR1	361
FGF/FGFR	FP-1039		Ligand trap	pan-FGF	362
	NSC12			pan-FGF	363
	SSR128129E		Allosteric	FGFRs	364
	AZD4547		TKI	FGFRs	365
	BGJ398			FGFR1-3	366
	LY287445			FGFRs	367
	FPA144		Antibody	FGFR2b	FivePrime
Ang/Tie2	Trebananib	AMG386	Peptibody	Ang-1/2	368
	Nesvacumab	REGN910	Antibody	Ang-2	369
	MEDI3617			Ang-2	370
	RO5520985			Bispecific: Ang-2 and VEGF	371
PDGF/FDGFR	Rinucumab	REGN2176-3	Antibody	PDGFR-β	372

(continued)

TABLE II (continues)

Angiogenic system	Molecule	Commercial name	Type of inhibitor	Target	Ref.
VEGF/VEGFR multitarget	Vatalanib	PTK787	TKI	VEGFR1-3, PDGFR-β, c-kit	373
	Pazopanib	Votrient		VEGFR1-3, PDGFR-α/β, FGFR1/3, c-kit	374
	Sorafenib	Nexavar		VEGFR2, FLT3, PDGFR, FGFR1	375
	Sunitinib	Sutent		c-kit, VEGFR1-3, PDGFR-α/β, FLT3, CSF-1R, RET	376
	Axitinib	Inlyta		VEGFR1-3	377
	Ponatinib	Iclusig		VEGFRs, BCR-ABL, FLT3, RET, c-kit, FGFRs, PDGFR	378
	Regorafenib	Stivarga		VEGFR1-3, RET, c-kit, PDGFR-α/β, FGFR1-2, and others	379
	Cabozantinib	Cabometyx		VEGFR2, c-Met	380
	Vandetanib	Caprelsa		VEGFRs, RET, EGFR	381
	Lenvatinib	Lenvima		VEGFRs, RET, FGFRs	382
	Nintedanib	BIBF1120		VEGFRs, PDGFR, FGFRs	383

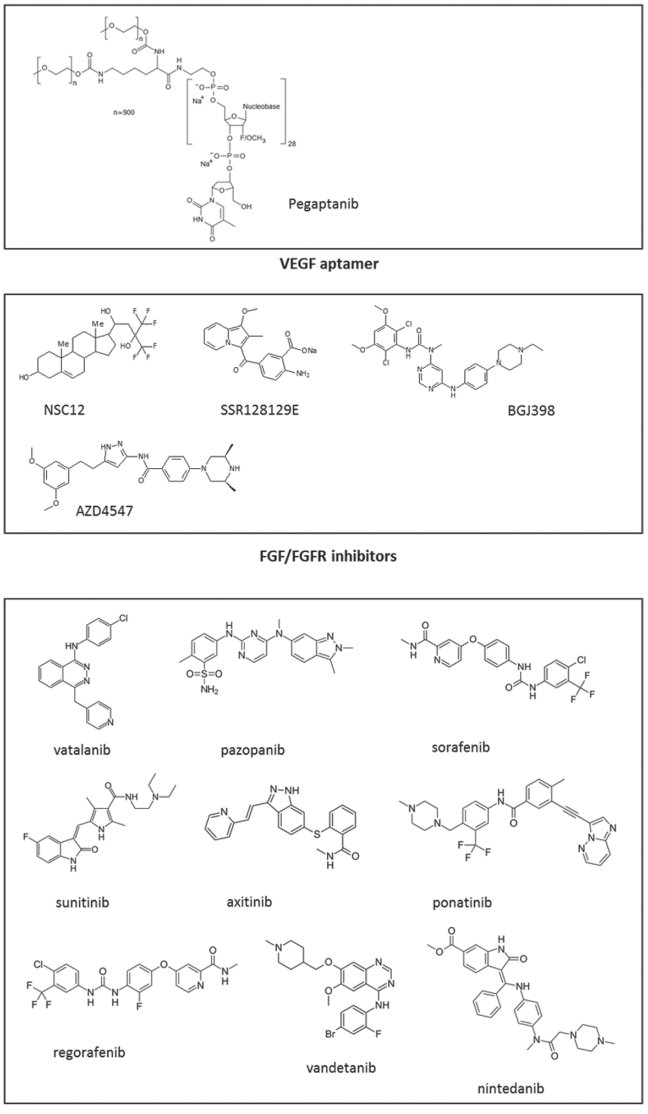

FIGURE 3 Chemical structures of FGF and VEGF inhibitors and multitarget RTKI.

agents. However, preclinical data provided evidence that cotargeting signaling by multiple proangiogenic factors is necessary to obtain an efficient and durable effect on tumor angiogenesis and growth, implying that multitarget inhibition might be a promising anticancer strategy. As an example, anti-VEGF therapy combined with the endogenous tumstatin peptide (that binds to $\alpha v\beta 3$ integrin) delayed tumor growth in human renal cell carcinoma xenografts only when administered together.[384]

Combination therapies with specific or multitargeting antiangiogenics may involve chemotherapy, radiotherapy, vascular-disrupting agents (VDAs), immunotherapy, or any other new approach. In particular, the use of chemotherapeutics together with antiangiogenic drugs has been widely explored and is actually a "hot topic" mainly in view of vascular normalization and metronomic chemotherapy (better described later herein).

VDAs target the already existing vessels in the tumor environment, thereby provoking a rapid collapse of the tumor vasculature leading to necrosis at the tumor core (reviewed in[385]). The clinical success of VDAs depends on the elimination of the viable tumor rim that is resistant to these compounds. This can be achieved by combination with

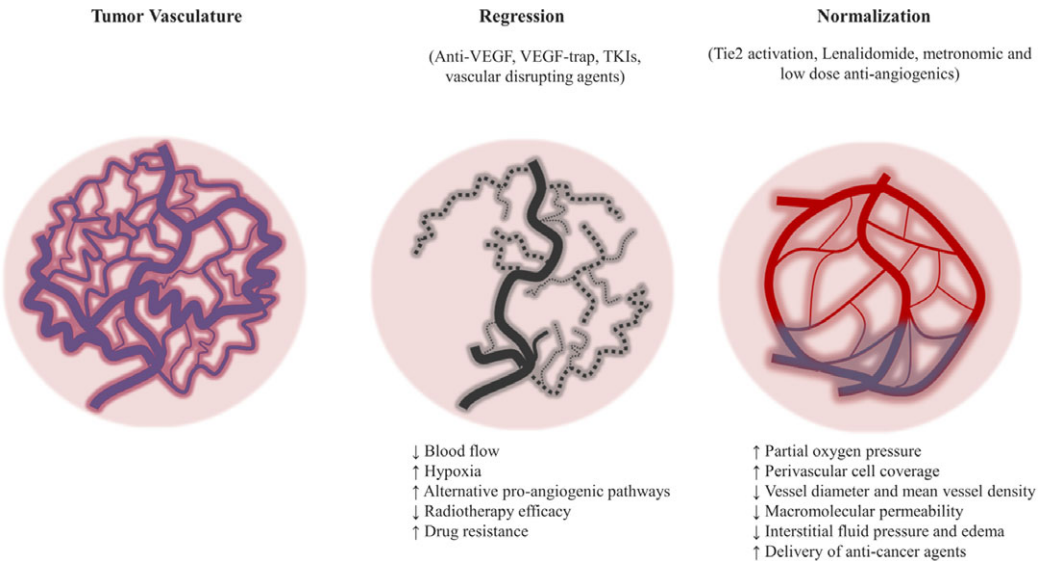

FIGURE 4 Features of vascular regression *vs* normalization. Graphical representation of PROs and CONs of antiangiogenic strategies that cause blood vessel regression or normalization in tumors. Examples of therapeutic agents are indicated between brackets.

radio/chemotherapy or antiangiogenic therapy, which may abrogate survival of the residual tumor cells and/or neovascularization from the remaining vessels in the tumor rim, respectively. A randomized phase II clinical trial evaluating the effect of bevacizumab *plus* fosbretabulin in recurrent ovarian, tubal, or peritoneal carcinoma reported encouraging results in terms of progression-free survival and overall response despite an increased risk of hypertension.[386]

Tumor angiogenesis also influences immune suppression, and angiogenic factors regulate immune cell trafficking across tumor endothelia.[387] For this reason targeting angiogenesis may be an effective approach to increase the efficacy of different types of immunotherapy, including immune checkpoint blockade.[388] Adoptive T-cell transfer in mice bearing large melanomas in combination with an anti-VEGF antibody significantly augmented antitumor activity and trafficking of T cells into the tumors.[389] The combination of CTLA4 blockade (ipilimumab) with bevacizumab improved survival of metastatic melanoma patients. This result was associated with endothelial activation and extensive $CD8^+$ and macrophage cell infiltration.[390] Several trials in melanoma are ongoing combining bevacizumab and immunotherapy with anti-PD1 (Nivolumab and MK-3475), anti-PDL1 (Atezolizumab), or anti-CTLA4 (Ipilimumab) antibodies.[390,391] In addition, the combination of anti-Ang-2 (MEDI3617) and anti-CTLA4 (tremelimumab) antibodies, as well as of pembrolizumab (anti-PD1) and Ziv-Aflibercept are in clinical evaluation for patients with melanoma or advanced solid tumors, respectively.[391]

These studies will provide the basis for further investigations regarding the interactions among angiogenic factors, blood vessel formation, immune regulation, and microenvironment, eventually paving the way for therapeutic exploitation of new drug combinations.

4.4.2 | Concept of vessel normalization versus regression

The tumor vasculature is characterized by increased vessel permeability and high interstitial fluid pressure as a consequence of its tortuous and chaotic structure, scarce pericyte coverage, and discontinuous BM. Instead of vascular disruption, vessel normalization aims to increase partial oxygen pressure and perivascular cell coverage in the tumor. This can be obtained through pruning and remodeling of abnormal tumor vessels, leading to vessels resembling normal tissue vasculature in terms of structure and function (Fig. 4).[9]

Preclinical studies have shown that anti-VEGF therapy and other antiangiogenic agents can induce vessel normalization.[10,392] In patients, bevacizumab monotherapy was found to induce an increase in pericyte coverage,

indicative of vascular normalization. Moreover, bevacizumab improved response to chemotherapy, especially in patients with a high pretreatment MVD, suggesting that this approach is only beneficial in highly vascularized tumors.[393]

Many examples of vascular normalization targets are available. Tie2 activation (by Ang-2-binding and Tie2-activating antibody) reduced tumor growth and metastasis by inducing tumor vascular normalization, increasing blood perfusion, and favoring chemotherapeutic drug delivery.[394] Dual inhibition of VEGFR/Ang-2 (using cediranib and MEDI3617) improved survival in orthotopic models of glioblastoma via enhanced vessel normalization and polarization of TAMs to antitumor phenotypes.[395] Pharmacological inhibition of the HGF receptor c-Met normalized vessels formed in vitro by GBM tumor-derived ECs.[396] Preclinical studies suggest that anti-VEGFR2 antibody (DC101) treatment can normalize orthotopic tumor vasculature and enhance the delivery of anticancer agents.[397,398]

Moreover, inhibition of oncogenic signaling may indirectly trigger vascular normalization. Blocking HER-2 signaling in breast cancer cells with the mAb trastuzumab (Herceptin) normalized breast tumor vessels by modulating the expression of at least four pro- and antiangiogenic molecules.[392] The early antitumor responses of castration in androgen-dependent carcinomas are primarily vascular effects due to an indirect mechanism where hormone depletion suppresses tumor cell production of angiogenic factors.[399] Suppression of BRAF signaling by PLX4720 diminished the expression of several proangiogenic factors downstream of BRAF, which in turn stabilized vessel architecture, improved perfusion, and abrogated hypoxia.[400] Recently, chloroquine has been shown to induce vascular normalization and reduce metastasis by enhancing Notch signaling in ECs.[401] Several other studies convincingly reported similar "normalizing" effects as a consequence of tumor cell oncogene (Ras, PI3K, AKT, EGFR) inhibition.[402]

These findings are supportive of the vascular normalization hypothesis proposed by Jain[61] that tumor blood vessels can revert from their abnormal phenotype to one more resembling normal tissues through restoration of the balance between pro- and antiangiogenic factors.

However, it should be taken into account that the benefits of vascular normalization are dose and time dependent.[403] Thus, optimizing combinations of antiangiogenic agents with anticancer treatments will require the assessment of imaging and/or circulating biomarkers to understand the dynamics of the tumor vascular response and the balance between vascular normalizing and antivascular effects.

4.4.3 | Additional targets and approaches

FGF/FGFR system. Additional angiogenesis regulators are emerging to target in combination with, or in response to, resistance to anti-VEGF blockade. One of these targets is represented by the FGF/FGFR system, which is activated in a variety of human tumors, leading to neovascularization, tumor progression, and metastatic dissemination. Efforts have been made to develop efficacious FGF/FGFR inhibitors for antiangiogenic/antitumor therapies (Fig. 3, Table II). These include selective and nonselective small-molecule TKIs, anti-FGFR antibodies, and some extracellular FGF ligand traps.[404,405]

Several wide-spectrum/nonselective TKIs block FGFRs with therapeutic efficacy (see[406,407] for more details). Regorafenib is a novel orally active multitarget compound that inhibits a number of proangiogenic RTKs, including FGFR1, VEGFR2, Tie2, and PDGFR.[408] Nintedanib (BIBF1120) interferes with VEGFR, PDGFR, and FGFR pathways.[409] All these multitargeting TKIs are endowed with toxicity profiles often related to their anti-VEGFR action.

Few selective FGFR inhibitors have been characterized and evaluated in clinical trials, like AZD4547 and LY287445 (pan-FGFR inhibitors) and BGJ398 (that targets FGFR1, FGFR2, and FGFR3). These new "FGFR-restricted" drugs show better tolerability compared to nonselective TKIs. Their most relevant side effects (hyperphosphatemia and tissue calcification) are strictly correlated to the inhibition of the FGF23 pathway. Apart from TKIs, the small-molecule SSR128129E binds extracellularly to FGFRs and inhibits FGFR signaling by an allosteric mechanism of action, without affecting orthosteric FGF binding.[364]

Finally, several FGF traps have been described. FP-1039 is a soluble FGFR1(IIIc)-Fc fusion protein that binds and inhibits almost all FGFs and has entered clinical trials,[362] whereas the long pentraxin-3 (PTX3)-derived small drug NSC12 represents the first low molecular weight FGF trap.[363] Interestingly, at variance with the hyperphosphatemic

effect of FGFR TKIs, administration of FGF traps (FP-1039 and NSC12) does not affect blood levels of phosphorus, calcium, and FGF23, and shows a safe profile in murine tumor models.

Hypoxia. Targeting hypoxic cells and inhibiting the HIF pathway is emerging as an important therapeutic strategy in cancer biology. Many compounds that target various other signaling pathways act also as indirect HIF inhibitors, including wortmannin, 2-methoxyestradiol and analogs, geldanamycin and analogs, radicicol and analogs, LYP294002, CCL-779FK-228, and others.[410]

Efforts have been made to identify direct HIF inhibitors. These agents are classified based on their ability to inhibit a particular step in the HIF pathway, such as HIF protein expression and stability, HIF dimerization, binding of HIF to target DNA sequences. Many of these small molecules have been evaluated as cancer therapeutics.[411] Chetomin disrupts the HIF-1α/p300 complex and shows antitumor activity in multiple myeloma patient-derived cell lines.[412] EZN-2968, an antagonist of HIF-1α mRNA impairs the growth of prostate cancer cells.[413] PX-12, which mediates HIF-1α ubiquitination and degradation by increasing spermidine/spermine acetyl transferase (SSAT2), displays antitumor activity in vitro and in vivo and is being evaluated in Phase II clinical trials.[414,415] Digoxin blocks HIF-1α protein synthesis, showed promising results in preclinical models of castrate-resistant prostate tumors, and is in clinical trial for prostate cancer.[416,417] Further clinical development of these hypoxia-targeting agents will require (i) improvement of direct and indirect methods to measure hypoxia in vivo and to select patients who could benefit from this therapy, as well as (ii) defining the appropriate treatment schedule.

Vascular targeting. An alternative therapeutic strategy to angiogenesis inhibitors, which block neovascularization, is represented by "vascular targeting,"[418] which relies on mAbs that recognize specific markers on newly formed blood vessels or in the stroma surrounding these vessels. These Abs selectively deliver potent therapeutic payloads (such as drugs, cytokines, radionuclides, photosensitizers, and toxins) to disease sites where neoangiogenesis takes place.[419,420]

Several antigens have been identified using immunohistochemical, proteomic, or transcriptomic screenings.[421,422] Well-known examples are represented by the alternatively spliced extradomains A and B of fibronectin, as well as the extradomain A1 of tenascin-C. These splice variants are undetectable in normal adult tissues but become strongly expressed at sites of physiological and tumor angiogenesis.[423,424] Another marker of angiogenesis, the lipid raft associated protein bone marrow stromal antigen-2 (Bst-2) appears to be more restricted to hematological malignancies like lymphomas.[425] To date, antibody-drug conjugates (ADC) and immunocytokines are advancing in clinical trials,[426] the latter with promising results, warranting efforts to precisely characterize their mechanisms of action.

Targeting EC metabolism. Recently, Carmeliet and co-workers postulated the concept that EC metabolism represents the engine of ECs, onto which proangiogenic signals (such as VEGF) converge.[427] As a consequence, whereas tumors develop resistance to treatment directed at specific proangiogenic proteins, targeting metabolic pathways may block the activation of ECs that are exposed to multiple angiogenic mediators (see also Section 3.4). The potential of this novel approach has recently been illustrated in several elegant studies.

Antiglycolytic therapy with a small molecule blocker of PFKFB3 (3PO) exerted convincing antiangiogenic effects in different in vitro and in vivo models. Interestingly, a partial and transient reduction of glycolysis by blockade of PFKFB3 seems to be sufficient to decrease pathological neovascularization.[278] Moreover, blockade (or genetic deficiency) of PFKFB3 results in inhibition of cancer cell invasion and metastasis. This effect is mainly due to increased tumor vessel normalization, improved vessel maturation, and perfusion. Indeed, PFKFB3 inhibition lowers the expression of cancer cell adhesion molecules in ECs, tightens the vascular barrier by reducing VE-cadherin endocytosis, and renders pericytes more quiescent and adhesive.[428]

In addition, recent findings identified FOXO1 as a major regulator of vascular activation. In particular, the FOXO1-MYC transcriptional axis emerged as a critical metabolic checkpoint for EC proliferation and as a new target for antiangiogenic therapies.[286]

Also FA metabolism and nucleotide synthesis might present potential therapeutic antiangiogenic targets. CPT1A silencing depletes EC stores of aspartate and dNTPs, and pharmacological blockade of CPT1 by etomoxir (an irreversible inhibitor of mitochondrial long-chain FAO) inhibits pathological ocular angiogenesis in mice.[289] Thus, targeting

EC metabolism represents an emerging therapeutic option as new targets are being identified and/or under characterization.

5 | CONCLUSION

Almost 50 years of angiogenesis research have resulted in increasingly more complex insights into tumor vascularization. These insights have provided novel hypotheses and concepts that may explain the disappointing anticancer efficacy of VEGF/VEGFR targeted agents in the clinic, and may pave the way for improved antiangiogenic cancer treatment.

First, a major part of the complexity in antiangiogenic therapy can be ascribed to the various mechanisms used by tumors to increase their blood supply (Fig. 1).[2] These modes of vascularization may coexist within a tumor,[60] but tumors may also shift from one mechanism to another during growth and metastasis, and in response to treatment.[75,309,321] Whereas sprouting angiogenesis has been investigated intensively, the study of these alternative modes of vascularization faces several problems. Cellular and molecular mediators of these vascularization mechanisms are poorly defined. Intratumoral vessels derived from VM are morphologically indistinguishable from normal blood vessels. Also, markers that unambiguously distinguish angiogenic versus co-opted vessels are lacking. The extent to which EPCs and CSCs contribute to tumor vascularization is unclear in part because of the lack of a consensus definition of these cell types. Because of these limitations, information is lacking regarding the relative contribution of blood vessels derived from these different vascularization modes in tumors.

A second obstacle to the clinical use of antiangiogenic drugs is the emergence of tumor resistance. Although antiangiogenic agents were anticipated to target genetically stable cells (i.e., ECs) that lack the capacity to rapidly adapt to treatment, various tumor escape mechanisms have been observed. Tumors respond to treatment with VEGF/VEGFR-targeted drugs by the expression and activation of alternative angiogenic factors and pathways.[292,294] Moreover, antiangiogenic treatment has been shown to select for hypoxia-tolerant[331] or vessel-independent tumor cells[323] or a tumor vasculature that arises in the absence of angiogenesis.[319] Thus, blocking angiogenesis may lead to the occurrence of more aggressive tumor cells and increased metastasis.

Third, the identification of novel mediators of angiogenesis has further diversified the angiogenesis landscape and has complicated target selection. Molecular and functional analyses of sprouting angiogenesis have uncovered a link between neuronal guidance and tip cell sprouting.[3,4] Similarly, anti- and proangiogenic functions have been ascribed to various proteins involved in bone homeostasis.[5] Emerging avenues further include the diverse functions elicited by microRNAs[6] and the role of EC metabolism in angiogenesis.[7] In particular, the latter is increasingly being recognized as a key determinant of angiogenesis regulation. Recent in vivo studies showed that a partial and transient reduction of the glycolytic enzyme PFKFB3 is sufficient to induce endothelial quiescence without affecting glycolysis required for normal cell maintenance.[278] This opens perspectives for the development of novel nontoxic antiangiogenic therapies. However, to date few metabolic enzymes have been studied in ECs. Research has mainly focused on the relation between a specific metabolic pathway and angiogenesis, and insights into the role of different metabolic pathways in angiogenesis are lacking. Moreover, the interactions between ECs and the tumor microenvironment should be considered. Stromal and tumor cells, but also viruses and bacteria that reside in the tumor microenvironment may compete with ECs for nutrient and/or metabolite usage.

The major question that remains is: how can the recently acquired knowledge be translated to improved anticancer efficacy of antiangiogenic agents? The answer lies not only in a better understanding of the mechanisms of action and resistance of currently used anti-VEGF agents, but also in a better selection of the patient population. Antiangiogenic drugs have predominantly been tested in unselected patients with large tumors. However, since these agents are in general not cytotoxic, the treatment of small tumors might provide more favorable results. Related to this point, therapy would greatly benefit from the identification of biomarkers that can predict which patients are likely to respond to a given therapy. Considering the multifactorial nature of neovascularization, which involves a variety of cell types and mediators, it is unlikely that targeting a single angiogenic factor will afford a protective effect. Future therapies should

tackle the process at different levels by using multitarget compounds or by blocking EC metabolism, which may overrule signals transmitted by various growth factors. Studies should also focus on the elucidation of the genetic basis of tumor vessel normalization, in order to prevent the selection of more aggressive tumor cell clones and to improve the delivery and/or activity of radio- and chemotherapy. Thereby, it should be taken into consideration that in the tumor, not only the blood vessels, but also other components of the tumor microenvironment are abnormal. Thus, therapeutic agents should normalize the entire tumor microenvironment, including cancer-associated fibroblasts and protumor immune cells. All together, these future insights may boost the development of totally new classes of compounds and/or treatment schedules with hopefully improved clinical anticancer efficacy.

REFERENCES

1. Liekens S, De Clercq E, Neyts J. Angiogenesis: Regulators and clinical applications. *Biochem Pharmacol* 2001;61(3):253–270.
2. Hillen F, Griffioen AW. Tumour vascularization: Sprouting angiogenesis and beyond. *Cancer Metastasis Rev* 2007;26(3–4):489–502.
3. Mehlen P, Delloye-Bourgeois C, Chedotal A. Novel roles for Slits and netrins: Axon guidance cues as anticancer targets? *Nat Rev Cancer* 2011;11(3):188–197.
4. Tamagnone L. Emerging role of semaphorins as major regulatory signals and potential therapeutic targets in cancer. *Cancer Cell* 2012;22(2):145–152.
5. Zhang L, Ye Y, Long X, Xiao P, Ren X, Yu J. BMP signaling and its paradoxical effects in tumorigenesis and dissemination. *Oncotarget* 2016;7(47):78206–78218.
6. Wang W, Zhang E, Lin C. MicroRNAs in tumor angiogenesis. *Life Sci* 2015;136:28–35.
7. Eelen G, de Zeeuw P, Simons M, Carmeliet P. Endothelial cell metabolism in normal and diseased vasculature. *Circ Res* 2015;116(7):1231–1244.
8. Ferrara N, Adamis AP. Ten years of anti-vascular endothelial growth factor therapy. *Nat Rev Drug Discov* 2016;15(6):385–403.
9. Carmeliet P, Jain RK. Principles and mechanisms of vessel normalization for cancer and other angiogenic diseases. *Nat Rev Drug Discov* 2011;10(6):417–427.
10. Arjaans M, Schroder CP, Oosting SF, Dafni U, Kleibeuker JE, de Vries EG. VEGF pathway targeting agents, vessel normalization and tumor drug uptake: From bench to bedside. *Oncotarget* 2016;7(16):21247–21258.
11. Folkman J. Tumor angiogenesis: Therapeutic implications. *N Engl J Med* 1971;285(21):1182–1186.
12. Welti J, Loges S, Dimmeler S, Carmeliet P. Recent molecular discoveries in angiogenesis and antiangiogenic therapies in cancer. *J Clin Invest* 2013;123(8):3190–3200.
13. Hlushchuk R, Makanya AN, Djonov V. Escape mechanisms after antiangiogenic treatment, or why are the tumors growing again? *Int J Dev Biol* 2011;55(4–5):563–567.
14. Ribatti D, Djonov V. Intussusceptive microvascular growth in tumors. *Cancer Lett* 2012;316(2):126–131.
15. Donnem T, Hu J, Ferguson M, et al. Vessel co-option in primary human tumors and metastases: An obstacle to effective anti-angiogenic treatment? *Cancer Med* 2013;2(4):427–436.
16. de la Puente P, Muz B, Azab F, Azab AK. Cell trafficking of endothelial progenitor cells in tumor progression. *Clin Cancer Res* 2013;19(13):3360–3368.
17. Moschetta M, Mishima Y, Sahin I, et al. Role of endothelial progenitor cells in cancer progression. *Biochim Biophys Acta* 2014;1846(1):26–39.
18. Seftor RE, Hess AR, Seftor EA, et al. Tumor cell vasculogenic mimicry: From controversy to therapeutic promise. *Am J Pathol* 2012;181(4):1115–1125.
19. Koch S, Claesson-Welsh L. Signal transduction by vascular endothelial growth factor receptors. *Cold Spring Harb Perspect Med* 2012;2(7):a006502.
20. Geudens I, Gerhardt H. Coordinating cell behaviour during blood vessel formation. *Development* 2011;138(21):4569–4583.
21. Hellstrom M, Phng LK, Hofmann JJ, et al. Dll4 signalling through Notch1 regulates formation of tip cells during angiogenesis. *Nature* 2007;445(7129):776–780.

22. Suchting S, Freitas C, le Noble F, et al. The Notch ligand Delta-like 4 negatively regulates endothelial tip cell formation and vessel branching. *Proc Natl Acad Sci USA* 2007;104(9):3225–3230.
23. Lobov IB, Renard RA, Papadopoulos N, et al. Delta-like ligand 4 (Dll4) is induced by VEGF as a negative regulator of angiogenic sprouting. *Proc Natl Acad Sci USA* 2007;104(9):3219–3224.
24. Harrington LS, Sainson RC, Williams CK, et al. Regulation of multiple angiogenic pathways by Dll4 and Notch in human umbilical vein endothelial cells. *Microvasc Res* 2008;75(2):144–154.
25. Funahashi Y, Shawber CJ, Vorontchikhina M, Sharma A, Outtz HH, Kitajewski J. Notch regulates the angiogenic response via induction of VEGFR-1. *J Angiogenes Res* 2010;2(1):3.
26. Krueger J, Liu D, Scholz K, et al. Flt1 acts as a negative regulator of tip cell formation and branching morphogenesis in the zebrafish embryo. *Development* 2011;138(10):2111–2120.
27. Jakobsson L, Franco CA, Bentley K, et al. Endothelial cells dynamically compete for the tip cell position during angiogenic sprouting. *Nat Cell Biol* 2010;12(10):943–953.
28. Siekmann AF, Lawson ND. Notch signalling limits angiogenic cell behaviour in developing zebrafish arteries. *Nature* 2007;445(7129):781–784.
29. Shawber CJ, Funahashi Y, Francisco E, et al. Notch alters VEGF responsiveness in human and murine endothelial cells by direct regulation of VEGFR-3 expression. *J Clin Invest* 2007;117(11):3369–3382.
30. Tammela T, Zarkada G, Wallgard E, et al. Blocking VEGFR-3 suppresses angiogenic sprouting and vascular network formation. *Nature* 2008;454(7204):656–660.
31. Benedito R, Rocha SF, Woeste M, et al. Notch-dependent VEGFR3 upregulation allows angiogenesis without VEGF-VEGFR2 signalling. *Nature* 2012;484(7392):110–114.
32. Thurston G, Kitajewski J. VEGF and Delta-Notch: Interacting signalling pathways in tumour angiogenesis. *Br J Cancer* 2008;99(8):1204–1209.
33. Noguera-Troise I, Daly C, Papadopoulos NJ, et al. Blockade of Dll4 inhibits tumour growth by promoting non-productive angiogenesis. *Nature* 2006;444(7122):1032–1037.
34. del Toro R, Prahst C, Mathivet T, et al. Identification and functional analysis of endothelial tip cell-enriched genes. *Blood* 2010;116(19):4025–4033.
35. Strasser GA, Kaminker JS, Tessier-Lavigne M. Microarray analysis of retinal endothelial tip cells identifies CXCR4 as a mediator of tip cell morphology and branching. *Blood* 2010;115(24):5102–5110.
36. Vandekeere S, Dewerchin M, Carmeliet P. Angiogenesis revisited: An overlooked role of endothelial cell metabolism in vessel sprouting. *Microcirculation* 2015;22(7):509–517.
37. Larrivee B, Freitas C, Suchting S, Brunet I, Eichmann A. Guidance of vascular development: Lessons from the nervous system. *Circ Res* 2009;104(4):428–441.
38. Fantin A, Vieira JM, Plein A, et al. NRP1 acts cell autonomously in endothelium to promote tip cell function during sprouting angiogenesis. *Blood* 2013;121(12):2352–2362.
39. Segarra M, Ohnuki H, Maric D, et al. Semaphorin 6A regulates angiogenesis by modulating VEGF signaling. *Blood* 2012;120(19):4104–4115.
40. Kim J, Oh WJ, Gaiano N, Yoshida Y, Gu C. Semaphorin 3E-Plexin-D1 signaling regulates VEGF function in developmental angiogenesis via a feedback mechanism. *Genes Dev* 2011;25(13):1399–1411.
41. Sawamiphak S, Seidel S, Essmann CL, et al. Ephrin-B2 regulates VEGFR2 function in developmental and tumour angiogenesis. *Nature* 2010;465(7297):487–491.
42. Wang Y, Nakayama M, Pitulescu ME, et al. Ephrin-B2 controls VEGF-induced angiogenesis and lymphangiogenesis. *Nature* 2010;465(7297):483–486.
43. Vestweber D, Broermann A, Schulte D. Control of endothelial barrier function by regulating vascular endothelial-cadherin. *Curr Opin Hematol* 2010;17(3):230–236.
44. Almagro S, Durmort C, Chervin-Petinot A, et al. The motor protein myosin-X transports VE-cadherin along filopodia to allow the formation of early endothelial cell-cell contacts. *Mol Cell Biol* 2010;30(7):1703–1717.
45. Fantin A, Vieira JM, Gestri G, et al. Tissue macrophages act as cellular chaperones for vascular anastomosis downstream of VEGF-mediated endothelial tip cell induction. *Blood* 2010;116(5):829–840.
46. Kubota Y, Takubo K, Shimizu T, et al. M-CSF inhibition selectively targets pathological angiogenesis and lymphangiogenesis. *J Exp Med* 2009;206(5):1089–1102.
47. Qian BZ, Pollard JW. Macrophage diversity enhances tumor progression and metastasis. *Cell* 2010;141(1):39–51.

48. Outtz HH, Tattersall IW, Kofler NM, Steinbach N, Kitajewski J. Notch1 controls macrophage recruitment and Notch signaling is activated at sites of endothelial cell anastomosis during retinal angiogenesis in mice. *Blood* 2011;118(12):3436–3439.
49. Serra H, Chivite I, Angulo-Urarte A, et al. PTEN mediates Notch-dependent stalk cell arrest in angiogenesis. *Nat Commun* 2015;6:7935.
50. Ishitani T, Matsumoto K, Chitnis AB, Itoh M. Nrarp functions to modulate neural-crest-cell differentiation by regulating LEF1 protein stability. *Nat Cell Biol* 2005;7(11):1106–1112.
51. Phng LK, Potente M, Leslie JD, et al. Nrarp coordinates endothelial Notch and Wnt signaling to control vessel density in angiogenesis. *Dev Cell* 2009;16(1):70–82.
52. Cooke VG, LeBleu VS, Keskin D, et al. Pericyte depletion results in hypoxia-associated epithelial-to-mesenchymal transition and metastasis mediated by met signaling pathway. *Cancer Cell* 2012;21(1):66–81.
53. Jain RK. Molecular regulation of vessel maturation. *Nat Med* 2003;9(6):685–693.
54. Caduff JH, Fischer LC, Burri PH. Scanning electron microscope study of the developing microvasculature in the postnatal rat lung. *Anat Rec* 1986;216(2):154–164.
55. Paku S, Dezso K, Bugyik E, et al. A new mechanism for pillar formation during tumor-induced intussusceptive angiogenesis: Inverse sprouting. *Am J Pathol* 2011;179(3):1573–1585.
56. Patan S, Munn LL, Jain RK. Intussusceptive microvascular growth in a human colon adenocarcinoma xenograft: A novel mechanism of tumor angiogenesis. *Microvasc Res* 1996;51(2):260–272.
57. Crivellato E, Nico B, Vacca A, Ribatti D. B-cell non-Hodgkin's lymphomas express heterogeneous patterns of neovascularization. *Haematologica* 2003;88(6):671–678.
58. Ribatti D, Nico B, Floris C, et al. Microvascular density, vascular endothelial growth factor immunoreactivity in tumor cells, vessel diameter and intussusceptive microvascular growth in primary melanoma. *Oncol Rep* 2005;14(1):81–84.
59. Nico B, Crivellato E, Guidolin D, et al. Intussusceptive microvascular growth in human glioma. *Clin Exp Med* 2010;10(2):93–98.
60. Djonov V, Hogger K, Sedlacek R, Laissue J, Draeger A. MMP-19: cellular localization of a novel metalloproteinase within normal breast tissue and mammary gland tumours. *J Pathol* 2001;195(2):147–155.
61. Jain RK. Normalization of tumor vasculature: An emerging concept in antiangiogenic therapy. *Science* 2005;307(5706):58–62.
62. Wilting J, Birkenhager R, Eichmann A, et al. VEGF121 induces proliferation of vascular endothelial cells and expression of flk-1 without affecting lymphatic vessels of chorioallantoic membrane. *Dev Biol* 1996;176(1):76–85.
63. Makanya AN, Hlushchuk R, Baum O, Velinov N, Ochs M, Djonov V. Microvascular endowment in the developing chicken embryo lung. *Am J Physiol Lung Cell Mol Physiol* 2007;292(5):L1136–1146.
64. Makanya AN, Stauffer D, Ribatti D, Burri PH, Djonov V. Microvascular growth, development, and remodeling in the embryonic avian kidney: The interplay between sprouting and intussusceptive angiogenic mechanisms. *Microsc Res Tech* 2005;66(6):275–288.
65. Patan S. TIE1 and TIE2 receptor tyrosine kinases inversely regulate embryonic angiogenesis by the mechanism of intussusceptive microvascular growth. *Microvasc Res* 1998;56(1):1–21.
66. Thurston G, Suri C, Smith K, et al. Leakage-resistant blood vessels in mice transgenically overexpressing angiopoietin-1. *Science* 1999;286(5449):2511–2514.
67. Crivellato E, Nico B, Vacca A, Djonov V, Presta M, Ribatti D. Recombinant human erythropoietin induces intussusceptive microvascular growth in vivo. *Leukemia* 2004;18(2):331–336.
68. Hlushchuk R, Ehrbar M, Reichmuth P, et al. Decrease in VEGF expression induces intussusceptive vascular pruning. *Arterioscler Thromb Vasc Biol* 2011;31(12):2836–2844.
69. Holash J, Maisonpierre PC, Compton D, et al. Vessel cooption, regression, and growth in tumors mediated by angiopoietins and VEGF. *Science* 1999;284(5422):1994–1998.
70. Holash J, Wiegand SJ, Yancopoulos GD. New model of tumor angiogenesis: Dynamic balance between vessel regression and growth mediated by angiopoietins and VEGF. *Oncogene* 1999;18(38):5356–5362.
71. Kusters B, Leenders WP, Wesseling P, et al. Vascular endothelial growth factor-A(165) induces progression of melanoma brain metastases without induction of sprouting angiogenesis. *Cancer Res* 2002;62(2):341–345.
72. Coelho AL, Gomes MP, Catarino RJ, et al. Angiogenesis in NSCLC: Is vessel co-option the trunk that sustains the branches? *Oncotarget* 2016.

73. Sardari Nia P, Colpaert C, Blyweert B, et al. Prognostic value of nonangiogenic and angiogenic growth patterns in non-small-cell lung cancer. *Br J Cancer* 2004;91(7):1293–1300.
74. Hu J, Bianchi F, Ferguson M, et al. Gene expression signature for angiogenic and nonangiogenic non-small-cell lung cancer. *Oncogene* 2005;24(7):1212–1219.
75. Bridgeman VL, Vermeulen PB, Foo S, et al. Vessel co-option is common in human lung metastases and mediates resistance to anti-angiogenic therapy in preclinical lung metastasis models. *J Pathol* 2017;241(3):362–374.
76. Vermeulen PB, Colpaert C, Salgado R, et al. Liver metastases from colorectal adenocarcinomas grow in three patterns with different angiogenesis and desmoplasia. *J Pathol* 2001;195(3):336–342.
77. Stessels F, Van den Eynden G, Van der Auwera I, et al. Breast adenocarcinoma liver metastases, in contrast to colorectal cancer liver metastases, display a non-angiogenic growth pattern that preserves the stroma and lacks hypoxia. *Br J Cancer* 2004;90(7):1429–1436.
78. Passalidou E, Trivella M, Singh N, et al. Vascular phenotype in angiogenic and non-angiogenic lung non-small cell carcinomas. *Br J Cancer* 2002;86(2):244–249.
79. Budde MD, Gold E, Jordan EK, Smith-Brown M, Frank JA. Phase contrast MRI is an early marker of micrometastatic breast cancer development in the rat brain. *NMR Biomed* 2012;25(5):726–736.
80. Marcola M, Rodrigues CE. Endothelial progenitor cells in tumor angiogenesis: Another brick in the wall. *Stem Cells Int* 2015;2015:832649.
81. Asahara T, Murohara T, Sullivan A, et al. Isolation of putative progenitor endothelial cells for angiogenesis. *Science* 1997;275(5302):964–967.
82. Asahara T, Masuda H, Takahashi T, et al. Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. *Circ Res* 1999;85(3):221–228.
83. Liekens S, Schols D, Hatse S. CXCL12-CXCR4 axis in angiogenesis, metastasis and stem cell mobilization. *Curr Pharm Des* 2010;16(35):3903–3920.
84. Jin H, Su J, Garmy-Susini B, Kleeman J, Varner J. Integrin alpha4beta1 promotes monocyte trafficking and angiogenesis in tumors. *Cancer Res* 2006;66(4):2146–2152.
85. Heissig B, Hattori K, Dias S, et al. Recruitment of stem and progenitor cells from the bone marrow niche requires MMP-9 mediated release of kit–ligand. *Cell* 2002;109(5):625–637.
86. Ceradini DJ, Kulkarni AR, Callaghan MJ, et al. Progenitor cell trafficking is regulated by hypoxic gradients through HIF-1 induction of SDF-1. *Nat Med* 2004;10(8):858–864.
87. Reyes M, Dudek A, Jahagirdar B, Koodie L, Marker PH, Verfaillie CM. Origin of endothelial progenitors in human postnatal bone marrow. *J Clin Invest* 2002;109(3):337–346.
88. Qin G, Ii M, Silver M, et al. Functional disruption of alpha4 integrin mobilizes bone marrow-derived endothelial progenitors and augments ischemic neovascularization. *J Exp Med* 2006;203(1):153–163.
89. Paulis YW, Soetekouw PM, Verheul HM, Tjan-Heijnen VC, Griffioen AW. Signalling pathways in vasculogenic mimicry. *Biochim Biophys Acta* 2010;1806(1):18–28.
90. Maniotis AJ, Folberg R, Hess A, et al. Vascular channel formation by human melanoma cells in vivo and in vitro: Vasculogenic mimicry. *Am J Pathol* 1999;155(3):739–752.
91. Mihic-Probst D, Ikenberg K, Tinguely M, et al. Tumor cell plasticity and angiogenesis in human melanomas. *PLoS One* 2012;7(3):e33571.
92. Soda Y, Marumoto T, Friedmann-Morvinski D, et al. Transdifferentiation of glioblastoma cells into vascular endothelial cells. *Proc Natl Acad Sci USA* 2011;108(11):4274–4280.
93. Postovit LM, Margaryan NV, Seftor EA, et al. Human embryonic stem cell microenvironment suppresses the tumorigenic phenotype of aggressive cancer cells. *Proc Natl Acad Sci USA* 2008;105(11):4329–4334.
94. Seftor EA, Meltzer PS, Schatteman GC, et al. Expression of multiple molecular phenotypes by aggressive melanoma tumor cells: Role in vasculogenic mimicry. *Crit Rev Oncol Hematol* 2002;44(1):17–27.
95. Seftor RE, Seftor EA, Koshikawa N, et al. Cooperative interactions of laminin 5 gamma2 chain, matrix metalloproteinase-2, and membrane type-1-matrix/metalloproteinase are required for mimicry of embryonic vasculogenesis by aggressive melanoma. *Cancer Res* 2001;61(17):6322–6327.
96. Hendrix MJ, Seftor EA, Meltzer PS, et al. Expression and functional significance of VE-cadherin in aggressive human melanoma cells: Role in vasculogenic mimicry. *Proc Natl Acad Sci USA* 2001;98(14):8018–8023.
97. Hess AR, Seftor EA, Gruman LM, Kinch MS, Seftor RE, Hendrix MJ. VE-cadherin regulates EphA2 in aggressive melanoma cells through a novel signaling pathway: Implications for vasculogenic mimicry. *Cancer Biol Ther* 2006;5(2):228–233.

98. Vartanian A, Stepanova E, Grigorieva I, et al. Melanoma vasculogenic mimicry capillary-like structure formation depends on integrin and calcium signaling. *Microcirculation* 2011;18(5):390–399.
99. Wang JY, Sun T, Zhao XL, et al. Functional significance of VEGF-a in human ovarian carcinoma: Role in vasculogenic mimicry. *Cancer Biol Ther* 2008;7(5):758–766.
100. Folkins C, Shaked Y, Man S, et al. Glioma tumor stem-like cells promote tumor angiogenesis and vasculogenesis via vascular endothelial growth factor and stromal-derived factor 1. *Cancer Res* 2009;69(18):7243–7251.
101. Wang R, Chadalavada K, Wilshire J, et al. Glioblastoma stem-like cells give rise to tumour endothelium. *Nature* 2010;468(7325):829–833.
102. Ricci-Vitiani L, Pallini R, Biffoni M, et al. Tumour vascularization via endothelial differentiation of glioblastoma stem-like cells. *Nature* 2010;468(7325):824–828.
103. Cheng L, Huang Z, Zhou W, et al. Glioblastoma stem cells generate vascular pericytes to support vessel function and tumor growth. *Cell* 2013;153(1):139–152.
104. Chen Q, Zhang H, Liu Y, et al. Endothelial cells are progenitors of cardiac pericytes and vascular smooth muscle cells. *Nat Commun* 2016;7:12422.
105. Zhang Y, Sun B, Zhao X, et al. Clinical significances and prognostic value of cancer stem-like cells markers and vasculogenic mimicry in renal cell carcinoma. *J Surg Oncol* 2013;108(6):414–419.
106. Liu TJ, Sun BC, Zhao XL, et al. $CD133^+$ cells with cancer stem cell characteristics associates with vasculogenic mimicry in triple-negative breast cancer. *Oncogene* 2013;32(5):544–553.
107. Francescone R, Scully S, Bentley B, et al. Glioblastoma-derived tumor cells induce vasculogenic mimicry through Flk-1 protein activation. *J Biol Chem* 2012;287(29):24821–24831.
108. Hardy KM, Kirschmann DA, Seftor EA, et al. Regulation of the embryonic morphogen Nodal by Notch4 facilitates manifestation of the aggressive melanoma phenotype. *Cancer Res* 2010;70(24):10340–10350.
109. Nelsen SM, Christian JL. Site-specific cleavage of BMP4 by furin, PC6, and PC7. *J Biol Chem* 2009;284(40):27157–27166.
110. Constam DB, Robertson EJ. Regulation of bone morphogenetic protein activity by pro domains and proprotein convertases. *J Cell Biol* 1999;144(1):139–149.
111. Mu Y, Gudey SK, Landstrom M. Non-Smad signaling pathways. *Cell Tissue Res* 2012;347(1):11–20.
112. Zhang YE. Non-Smad pathways in TGF-beta signaling. *Cell Res* 2009;19(1):128–139.
113. Lowery JW, de Caestecker MP. BMP signaling in vascular development and disease. *Cytokine Growth Factor Rev* 2010;21(4):287–298.
114. Samad TA, Rebbapragada A, Bell E, et al. DRAGON, a bone morphogenetic protein co-receptor. *J Biol Chem* 2005;280(14):14122–14129.
115. Onichtchouk D, Chen YG, Dosch R, et al. Silencing of TGF-beta signalling by the pseudoreceptor BAMBI. *Nature* 1999;401(6752):480–485.
116. Kirkbride KC, Townsend TA, Bruinsma MW, Barnett JV, Blobe GC. Bone morphogenetic proteins signal through the transforming growth factor-beta type III receptor. *J Biol Chem* 2008;283(12):7628–7637.
117. Lee NY, Kirkbride KC, Sheu RD, Blobe GC. The transforming growth factor-beta type III receptor mediates distinct subcellular trafficking and downstream signaling of activin-like kinase (ALK)3 and ALK6 receptors. *Mol Biol Cell* 2009;20(20):4362–4370.
118. David L, Feige JJ, Bailly S. Emerging role of bone morphogenetic proteins in angiogenesis. *Cytokine Growth Factor Rev* 2009;20(3):203–212.
119. Wiley DM, Jin SW. Bone morphogenetic protein functions as a context-dependent angiogenic cue in vertebrates. *Semin Cell Dev Biol* 2011;22(9):1012–1018.
120. Finkenzeller G, Hager S, Stark GB. Effects of bone morphogenetic protein 2 on human umbilical vein endothelial cells. *Microvasc Res* 2012;84(1):81–85.
121. Valdimarsdottir G, Goumans MJ, Rosendahl A, et al. Stimulation of Id1 expression by bone morphogenetic protein is sufficient and necessary for bone morphogenetic protein-induced activation of endothelial cells. *Circulation* 2002;106(17):2263–2270.
122. Stabile H, Mitola S, Moroni E, et al. Bone morphogenic protein antagonist Drm/gremlin is a novel proangiogenic factor. *Blood* 2007;109(5):1834–1840.
123. Rothhammer T, Bataille F, Spruss T, Eissner G, Bosserhoff AK. Functional implication of BMP4 expression on angiogenesis in malignant melanoma. *Oncogene* 2007;26(28):4158–4170.

124. Yeh LC, Lee JC. Osteogenic protein-1 increases gene expression of vascular endothelial growth factor in primary cultures of fetal rat calvaria cells. *Mol Cell Endocrinol* 1999;153(1–2):113–124.
125. Deckers MM, van Bezooijen RL, van der Horst G, et al. Bone morphogenetic proteins stimulate angiogenesis through osteoblast-derived vascular endothelial growth factor A. *Endocrinology* 2002;143(4):1545–1553.
126. Johnson MD, Reeder JE, O'Connell M. Bone morphogenetic protein-4 and 7 and receptors regulate vascular endothelial growth factor and receptors in human fetal leptomeninges. *Neurosci Lett* 2015;606:225–230.
127. Bidart M, Ricard N, Levet S, et al. BMP9 is produced by hepatocytes and circulates mainly in an active mature form complexed to its prodomain. *Cell Mol Life Sci* 2012;69(2):313–324.
128. Rostama B, Turner JE, Seavey GT, et al. DLL4/Notch1 and BMP9 interdependent signaling induces human endothelial cell quiescence via P27KIP1 and thrombospondin-1. *Arterioscler Thromb Vasc Biol* 2015;35(12):2626–2637.
129. Laux DW, Young S, Donovan JP, Mansfield CJ, Upton PD, Roman BL. Circulating Bmp10 acts through endothelial Alk1 to mediate flow-dependent arterial quiescence. *Development* 2013;140(16):3403–3412.
130. David L, Mallet C, Keramidas M, et al. Bone morphogenetic protein-9 is a circulating vascular quiescence factor. *Circ Res* 2008;102(8):914–922.
131. Chen H, Brady Ridgway J, Sai T, et al. Context-dependent signaling defines roles of BMP9 and BMP10 in embryonic and postnatal development. *Proc Natl Acad Sci USA* 2013;110(29):11887–11892.
132. Ricard N, Ciais D, Levet S, et al. BMP9 and BMP10 are critical for postnatal retinal vascular remodeling. *Blood* 2012;119(25):6162–6171.
133. Scharpfenecker M, van Dinther M, Liu Z, et al. BMP-9 signals via ALK1 and inhibits bFGF-induced endothelial cell proliferation and VEGF-stimulated angiogenesis. *J Cell Sci* 2007;120(Pt 6):964–972.
134. Ntumba K, Akla N, Oh SP, Eichmann A, Larrivee B. BMP9/ALK1 inhibits neovascularization in mouse models of age-related macular degeneration. *Oncotarget* 2016;7(35):55957–55969.
135. de Vinuesa AG, Bocci M, Pietras K, Ten Dijke P. Targeting tumour vasculature by inhibiting activin receptor-like kinase (ALK)1 function. *Biochem Soc Trans* 2016;44(4):1142–1149.
136. Jonker L. TGF-beta & BMP receptors endoglin and ALK1: Overview of their functional role and status as antiangiogenic targets. *Microcirculation* 2014;21(2):93–103.
137. Hu-Lowe DD, Chen E, Zhang L, et al. Targeting activin receptor-like kinase 1 inhibits angiogenesis and tumorigenesis through a mechanism of action complementary to anti-VEGF therapies. *Cancer Res* 2011;71(4):1362–1373.
138. Voss MH, Bhatt RS, Plimack ER, et al. The DART Study: Results from the dose-escalation and expansion cohorts evaluating the combination of dalantercept plus axitinib in advanced renal cell carcinoma. *Clin Cancer Res* 2016. https://doi.org/10.1158/1078-0432.CCR-16-2395. [Epub ahead of print].
139. Jimeno A, Posner MR, Wirth LJ, et al. A phase 2 study of dalantercept, an activin receptor-like kinase-1 ligand trap, in patients with recurrent or metastatic squamous cell carcinoma of the head and neck. *Cancer* 2016;122(23):3641–3649.
140. Hawinkels LJ, de Vinuesa AG, Paauwe M, et al. Activin receptor-like kinase 1 ligand trap reduces microvascular density and improves chemotherapy efficiency to various solid tumors. *Clin Cancer Res* 2016;22(1):96–106.
141. Wheatley-Price P, Chu Q, Bonomi M, et al. A phase II study of PF-03446962 in patients with advanced malignant pleural mesothelioma. CCTG Trial IND.207. *J Thorac Oncol* 2016;11(11):2018–2021.
142. Goff LW, Cohen RB, Berlin JD, et al. A phase I study of the anti-activin receptor-like kinase 1 (ALK-1) monoclonal antibody PF-03446962 in patients with advanced solid tumors. *Clin Cancer Res* 2016;22(9):2146–2154.
143. van Meeteren LA, Thorikay M, Bergqvist S, et al. Anti-human activin receptor-like kinase 1 (ALK1) antibody attenuates bone morphogenetic protein 9 (BMP9)-induced ALK1 signaling and interferes with endothelial cell sprouting. *J Biol Chem* 2012;287(22):18551–18561.
144. Freitas C, Larrivee B, Eichmann A. Netrins and UNC5 receptors in angiogenesis. *Angiogenesis* 2008;11(1):23–29.
145. Ding Q, Liao SJ, Yu J. Axon guidance factor netrin-1 and its receptors regulate angiogenesis after cerebral ischemia. *Neurosci Bull* 2014;30(4):683–691.
146. Ballard MS, Hinck L. A roundabout way to cancer. *Adv Cancer Res* 2012;114:187–235.
147. Nguyen Ba-Charvet KT, Brose K, Ma L, et al. Diversity and specificity of actions of Slit2 proteolytic fragments in axon guidance. *J Neurosci* 2001;21(12):4281–4289.
148. Gohrig A, Detjen KM, Hilfenhaus G, et al. Axon guidance factor SLIT2 inhibits neural invasion and metastasis in pancreatic cancer. *Cancer Res* 2014;74(5):1529–1540.

149. Zhang B, Xiao W, Qiu H, et al. Heparan sulfate deficiency disrupts developmental angiogenesis and causes congenital diaphragmatic hernia. *J Clin Invest* 2014;124(1):209–221.

150. Sheldon H, Andre M, Legg JA, et al. Active involvement of Robo1 and Robo4 in filopodia formation and endothelial cell motility mediated via WASP and other actin nucleation-promoting factors. *FASEB J* 2009;23(2):513–522.

151. Koch AW, Mathivet T, Larrivee B, et al. Robo4 maintains vessel integrity and inhibits angiogenesis by interacting with UNC5B. *Dev Cell* 2011;20(1):33–46.

152. Zhang F, Prahst C, Mathivet T, et al. The Robo4 cytoplasmic domain is dispensable for vascular permeability and neovascularization. *Nat Commun* 2016;7:13517.

153. Hu H. Cell-surface heparan sulfate is involved in the repulsive guidance activities of Slit2 protein. *Nat Neurosci* 2001;4(7):695–701.

154. de Wit J, Verhaagen J. Proteoglycans as modulators of axon guidance cue function. *Adv Exp Med Biol* 2007;600:73–89.

155. Marlow R, Binnewies M, Sorensen LK, et al. Vascular Robo4 restricts proangiogenic VEGF signaling in breast. *Proc Natl Acad Sci USA* 2010;107(23):10520–10525.

156. Jones CA, London NR, Chen H, et al. Robo4 stabilizes the vascular network by inhibiting pathologic angiogenesis and endothelial hyperpermeability. *Nat Med* 2008;14(4):448–453.

157. Jones CA, Nishiya N, London NR, et al. Slit2-Robo4 signalling promotes vascular stability by blocking Arf6 activity. *Nat Cell Biol* 2009;11(11):1325–1331.

158. Pircher A, Fiegl M, Untergasser G, et al. Favorable prognosis of operable non-small cell lung cancer (NSCLC) patients harboring an increased expression of tumor endothelial markers (TEMs). *Lung Cancer* 2013;81(2):252–258.

159. Rama N, Dubrac A, Mathivet T, et al. Slit2 signaling through Robo1 and Robo2 is required for retinal neovascularization. *Nat Med* 2015;21(5):483–491.

160. Wang B, Xiao Y, Ding BB, et al. Induction of tumor angiogenesis by Slit-Robo signaling and inhibition of cancer growth by blocking Robo activity. *Cancer Cell* 2003;4(1):19–29.

161. Wang LJ, Zhao Y, Han B, et al. Targeting Slit-Roundabout signaling inhibits tumor angiogenesis in chemical-induced squamous cell carcinogenesis. *Cancer Sci* 2008;99(3):510–517.

162. Dallol A, Da Silva NF, Viacava P, et al. SLIT2, a human homologue of the Drosophila Slit2 gene, has tumor suppressor activity and is frequently inactivated in lung and breast cancers. *Cancer Res* 2002;62(20):5874–5880.

163. Park KW, Morrison CM, Sorensen LK, et al. Robo4 is a vascular-specific receptor that inhibits endothelial migration. *Dev Biol* 2003;261(1):251–267.

164. Nguyen-Ba-Charvet KT, Brose K, Marillat V, Sotelo C, Tessier-Lavigne M, Chedotal A. Sensory axon response to substrate-bound Slit2 is modulated by laminin and cyclic GMP. *Mol Cell Neurosci* 2001;17(6):1048–1058.

165. Dunaway CM, Hwang Y, Lindsley CW, et al. Cooperative signaling between Slit2 and Ephrin-A1 regulates a balance between angiogenesis and angiostasis. *Mol Cell Biol* 2011;31(3):404–416.

166. Neufeld G, Mumblat Y, Smolkin T, et al. The role of the semaphorins in cancer. *Cell Adh Migr* 2016;10(6):652–674.

167. Klostermann A, Lohrum M, Adams RH, Puschel AW. The chemorepulsive activity of the axonal guidance signal semaphorin D requires dimerization. *J Biol Chem* 1998;273(13):7326–7331.

168. Janssen BJ, Robinson RA, Perez-Branguli F, et al. Structural basis of semaphorin-plexin signalling. *Nature* 2010;467(7319):1118–1122.

169. Nogi T, Yasui N, Mihara E, et al. Structural basis for semaphorin signalling through the plexin receptor. *Nature* 2010;467(7319):1123–1127.

170. Battistini C, Tamagnone L. Transmembrane semaphorins, forward and reverse signaling: Have a look both ways. *Cell Mol Life Sci* 2016;73(8):1609–1622.

171. Bates D, Taylor GI, Minichiello J, et al. Neurovascular congruence results from a shared patterning mechanism that utilizes Semaphorin3A and Neuropilin-1. *Dev Biol* 2003;255(1):77–98.

172. Serini G, Valdembri D, Zanivan S, et al. Class 3 semaphorins control vascular morphogenesis by inhibiting integrin function. *Nature* 2003;424(6947):391–397.

173. Maione F, Molla F, Meda C, et al. Semaphorin 3A is an endogenous angiogenesis inhibitor that blocks tumor growth and normalizes tumor vasculature in transgenic mouse models. *J Clin Invest* 2009;119(11):3356–3372.

174. Li K, Chen MK, Li LY, et al. The predictive value of semaphorins 3 expression in biopsies for biochemical recurrence of patients with low- and intermediate-risk prostate cancer. *Neoplasma* 2013;60(6):683–689.

175. Staton CA, Shaw LA, Valluru M, et al. Expression of class 3 semaphorins and their receptors in human breast neoplasia. *Histopathology* 2011;59(2):274–282.
176. Bielenberg DR, Hida Y, Shimizu A, et al. Semaphorin 3F, a chemorepulsant for endothelial cells, induces a poorly vascularized, encapsulated, nonmetastatic tumor phenotype. *J Clin Invest* 2004;114(9):1260–1271.
177. Kusy S, Nasarre P, Chan D, et al. Selective suppression of in vivo tumorigenicity by semaphorin SEMA3F in lung cancer cells. *Neoplasia* 2005;7(5):457–465.
178. Casazza A, Fu X, Johansson I, et al. Systemic and targeted delivery of semaphorin 3A inhibits tumor angiogenesis and progression in mouse tumor models. *Arterioscler Thromb Vasc Biol* 2011;31(4):741–749.
179. Holmes S, Downs AM, Fosberry A, et al. Sema7A is a potent monocyte stimulator. *Scand J Immunol* 2002;56(3):270–275.
180. Meda C, Molla F, De Pizzol M, et al. Semaphorin 4A exerts a proangiogenic effect by enhancing vascular endothelial growth factor-A expression in macrophages. *J Immunol* 2012;188(8):4081–4092.
181. Casazza A, Laoui D, Wenes M, et al. Impeding macrophage entry into hypoxic tumor areas by Sema3A/Nrp1 signaling blockade inhibits angiogenesis and restores antitumor immunity. *Cancer Cell* 2013;24(6):695–709.
182. Sierra JR, Corso S, Caione L, et al. Tumor angiogenesis and progression are enhanced by Sema4D produced by tumor-associated macrophages. *J Exp Med* 2008;205(7):1673–1685.
183. Conrotto P, Valdembri D, Corso S, et al. Sema4D induces angiogenesis through Met recruitment by Plexin B1. *Blood* 2005;105(11):4321–4329.
184. Lu Y, Xu Q, Chen L, et al. Expression of semaphorin 6D and its receptor plexin-A1 in gastric cancer and their association with tumor angiogenesis. *Oncol Lett* 2016;12(5):3967–3974.
185. Barquilla A, Pasquale EB. Eph receptors and ephrins: Therapeutic opportunities. *Annu Rev Pharmacol Toxicol* 2015;55:465–487.
186. Salvucci O, Tosato G. Essential roles of EphB receptors and EphrinB ligands in endothelial cell function and angiogenesis. *Adv Cancer Res* 2012;114:21–57.
187. Lisle JE, Mertens-Walker I, Rutkowski R, Herington AC, Stephenson SA. Eph receptors and their ligands: Promising molecular biomarkers and therapeutic targets in prostate cancer. *Biochim Biophys Acta* 2013;1835(2):243–257.
188. Wang HU, Chen ZF, Anderson DJ. Molecular distinction and angiogenic interaction between embryonic arteries and veins revealed by ephrin-B2 and its receptor Eph-B4. *Cell* 1998;93(5):741–753.
189. Gerety SS, Anderson DJ. Cardiovascular ephrinB2 function is essential for embryonic angiogenesis. *Development* 2002;129(6):1397–1410.
190. Makinen T, Adams RH, Bailey J, et al. PDZ interaction site in ephrinB2 is required for the remodeling of lymphatic vasculature. *Genes Dev* 2005;19(3):397–410.
191. Zhang G, Brady J, Liang WC, Wu Y, Henkemeyer M, Yan M. EphB4 forward signalling regulates lymphatic valve development. *Nat Commun* 2015;6:6625.
192. Foo SS, Turner CJ, Adams S, et al. Ephrin-B2 controls cell motility and adhesion during blood-vessel-wall assembly. *Cell* 2006;124(1):161–173.
193. Amato KR, Wang S, Hastings AK, et al. Genetic and pharmacologic inhibition of EPHA2 promotes apoptosis in NSCLC. *J Clin Invest* 2014;124(5):2037–2049.
194. Brantley-Sieders DM, Zhuang G, Hicks D, et al. The receptor tyrosine kinase EphA2 promotes mammary adenocarcinoma tumorigenesis and metastatic progression in mice by amplifying ErbB2 signaling. *J Clin Invest* 2008;118(1):64–78.
195. Pasquale EB. Eph-ephrin bidirectional signaling in physiology and disease. *Cell* 2008;133(1):38–52.
196. Larson J, Schomberg S, Schroeder W, Carpenter TC. Endothelial EphA receptor stimulation increases lung vascular permeability. *Am J Physiol Lung Cell Mol Physiol* 2008;295(3):L431–L439.
197. Brantley-Sieders DM, Fang WB, Hicks DJ, Zhuang G, Shyr Y, Chen J. Impaired tumor microenvironment in EphA2-deficient mice inhibits tumor angiogenesis and metastatic progression. *FASEB J* 2005;19(13):1884–1886.
198. Castellvi J, Garcia A, de la Torre J, et al. Ephrin B expression in epithelial ovarian neoplasms correlates with tumor differentiation and angiogenesis. *Hum Pathol* 2006;37(7):883–889.
199. Goparaju C, Donington JS, Hsu T, Harrington R, Hirsch N, Pass HI. Overexpression of EPH receptor B2 in malignant mesothelioma correlates with oncogenic behavior. *J Thorac Oncol* 2013;8(9):1203–1211.
200. Noren NK, Lu M, Freeman AL, Koolpe M, Pasquale EB. Interplay between EphB4 on tumor cells and vascular ephrin-B2 regulates tumor growth. *Proc Natl Acad Sci USA* 2004;101(15):5583–5588.

201. Kertesz N, Krasnoperov V, Reddy R, et al. The soluble extracellular domain of EphB4 (sEphB4) antagonizes EphB4-EphrinB2 interaction, modulates angiogenesis, and inhibits tumor growth. *Blood* 2006;107(6):2330–2338.
202. Martiny-Baron G, Korff T, Schaffner F, et al. Inhibition of tumor growth and angiogenesis by soluble EphB4. *Neoplasia* 2004;6(3):248–257.
203. Noren NK, Foos G, Hauser CA, Pasquale EB. The EphB4 receptor suppresses breast cancer cell tumorigenicity through an Abl-Crk pathway. *Nat Cell Biol* 2006;8(8):815–825.
204. Lagos-Quintana M, Rauhut R, Lendeckel W, Tuschl T. Identification of novel genes coding for small expressed RNAs. *Science* 2001;294(5543):853–858.
205. Ambros V. The functions of animal microRNAs. *Nature* 2004;431(7006):350–355.
206. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 2004;116(2):281–297.
207. Stark A, Brennecke J, Bushati N, Russell RB, Cohen SM. Animal MicroRNAs confer robustness to gene expression and have a significant impact on 3′UTR evolution. *Cell* 2005;123(6):1133–1146.
208. Flores-Perez A, Marchat LA, Rodriguez-Cuevas S, et al. Dual targeting of ANGPT1 and TGFBR2 genes by miR-204 controls angiogenesis in breast cancer. *Sci Rep* 2016;6:34504.
209. He T, Qi F, Jia L, et al. MicroRNA-542-3p inhibits tumour angiogenesis by targeting angiopoietin-2. *J Pathol* 2014; 232(5):499–508.
210. Bridge G, Monteiro R, Henderson S, et al. The microRNA-30 family targets DLL4 to modulate endothelial cell behavior during angiogenesis. *Blood* 2012;120(25):5063–5072.
211. Jiang Q, Lagos-Quintana M, Liu D, et al. miR-30a regulates endothelial tip cell formation and arteriolar branching. *Hypertension* 2013;62(3):592–598.
212. Liu CH, Sun Y, Li J, et al. Endothelial microRNA-150 is an intrinsic suppressor of pathologic ocular neovascularization. *Proc Natl Acad Sci USA* 2015;112(39):12163–12168.
213. Zhou B, Ma R, Si W, et al. MicroRNA-503 targets FGF2 and VEGFA and inhibits tumor angiogenesis and growth. *Cancer Lett* 2013;333(2):159–169.
214. Yin KJ, Olsen K, Hamblin M, Zhang J, Schwendeman SP, Chen YE. Vascular endothelial cell-specific microRNA-15a inhibits angiogenesis in hindlimb ischemia. *J Biol Chem* 2012;287(32):27055–27064.
215. Zhang X, Xu J, Jiang T, Liu G, Wang D, Lu Y. MicroRNA-195 suppresses colorectal cancer cells proliferation via targeting FGF2 and regulating Wnt/beta-catenin pathway. *Am J Cancer Res* 2016;6(11):2631–2640.
216. Cheng Z, Ma R, Tan W, Zhang L. MiR-152 suppresses the proliferation and invasion of NSCLC cells by inhibiting FGF2. *Exp Mol Med* 2014;46:e112.
217. He Q, Ren X, Chen J, et al. miR-16 targets fibroblast growth factor 2 to inhibit NPC cell proliferation and invasion via PI3K/AKT and MAPK signaling pathways. *Oncotarget* 2016;7(3):3047–3058.
218. Xu J, Wang T, Cao Z, et al. MiR-497 downregulation contributes to the malignancy of pancreatic cancer and associates with a poor prognosis. *Oncotarget* 2014;5(16):6983–6993.
219. Sun XH, Geng XL, Zhang J, Zhang C. miRNA-646 suppresses osteosarcoma cell metastasis by downregulating fibroblast growth factor 2 (FGF2). *Tumour Biol* 2015;36(3):2127–2134.
220. Hu Y, Qiu Y, Yague E, Ji W, Liu J, Zhang J. miRNA-205 targets VEGFA and FGF2 and regulates resistance to chemotherapeutics in breast cancer. *Cell Death Dis* 2016;7(6):e2291.
221. Yamakuchi M, Lotterman CD, Bao C, et al. P53-induced microRNA-107 inhibits HIF-1 and tumor angiogenesis. *Proc Natl Acad Sci USA* 2010;107(14):6334–6339.
222. Umezu T, Tadokoro H, Azuma K, Yoshizawa S, Ohyashiki K, Ohyashiki JH. Exosomal miR-135b shed from hypoxic multiple myeloma cells enhances angiogenesis by targeting factor-inhibiting HIF-1. *Blood* 2014;124(25):3748–3757.
223. Ma H, Pan JS, Jin LX, et al. MicroRNA-17∼92 inhibits colorectal cancer progression by targeting angiogenesis. *Cancer Lett* 2016;376(2):293–302.
224. Wu Z, Cai X, Huang C, Xu J, Liu A. miR-497 suppresses angiogenesis in breast carcinoma by targeting HIF-1alpha. *Oncol Rep* 2016;35(3):1696–1702.
225. Cha ST, Chen PS, Johansson G, et al. MicroRNA-519c suppresses hypoxia-inducible factor-1alpha expression and tumor angiogenesis. *Cancer Res* 2010;70(7):2675–2685.
226. Bartoszewska S, Kochan K, Piotrowski A, et al. The hypoxia-inducible miR-429 regulates hypoxia-inducible factor-1alpha expression in human endothelial cells through a negative feedback loop. *FASEB J* 2015;29(4):1467–1479.

227. Li Y, Kuscu C, Banach A, et al. miR-181a-5p inhibits cancer cell migration and angiogenesis via downregulation of matrix metalloproteinase-14. *Cancer Res* 2015;75(13):2674–2685.
228. Zheng L, Jiao W, Mei H, et al. miRNA-337-3p inhibits gastric cancer progression through repressing myeloid zinc finger 1-facilitated expression of matrix metalloproteinase 14. *Oncotarget* 2016;7(26):40314–40328.
229. Xiang X, Mei H, Zhao X, et al. miRNA-337-3p suppresses neuroblastoma progression by repressing the transcription of matrix metalloproteinase 14. *Oncotarget* 2015;6(26):22452–22466.
230. Xiang X, Mei H, Qu H, et al. miRNA-584-5p exerts tumor suppressive functions in human neuroblastoma through repressing transcription of matrix metalloproteinase 14. *Biochim Biophys Acta* 2015;1852(9):1743–1754.
231. Zhang H, Qi M, Li S, et al. microRNA-9 targets matrix metalloproteinase 14 to inhibit invasion, metastasis, and angiogenesis of neuroblastoma cells. *Mol Cancer Ther* 2012;11(7):1454–1466.
232. Fiedler J, Thum T. New insights into miR-17-92 cluster regulation and angiogenesis. *Circ Res* 2016;118(1):9–11.
233. Bao L, Yan Y, Xu C, et al. MicroRNA-21 suppresses PTEN and hSulf-1 expression and promotes hepatocellular carcinoma progression through AKT/ERK pathways. *Cancer Lett* 2013;337(2):226–236.
234. Wang J, Wang Y, Wang Y, et al. Transforming growth factor beta-regulated microRNA-29a promotes angiogenesis through targeting the phosphatase and tensin homolog in endothelium. *J Biol Chem* 2013;288(15):10418–10426.
235. Seok JK, Lee SH, Kim MJ, Lee YM. MicroRNA-382 induced by HIF-1alpha is an angiogenic miR targeting the tumor suppressor phosphatase and tensin homolog. *Nucleic Acids Res* 2014;42(12):8062–8072.
236. Nip H, Dar AA, Saini S, et al. Oncogenic microRNA-4534 regulates PTEN pathway in prostate cancer. *Oncotarget* 2016;7(42):68371–68384.
237. Mao G, Liu Y, Fang X, et al. Tumor-derived microRNA-494 promotes angiogenesis in non-small cell lung cancer. *Angiogenesis* 2015;18(3):373–382.
238. Liu LZ, Li C, Chen Q, et al. MiR-21 induced angiogenesis through AKT and ERK activation and HIF-1alpha expression. *PLoS One* 2011;6(4):e19139.
239. Sundaram P, Hultine S, Smith LM, et al. p53-responsive miR-194 inhibits thrombospondin-1 and promotes angiogenesis in colon cancers. *Cancer Res* 2011;71(24):7490–7501.
240. Feng N, Wang Z, Zhang Z, He X, Wang C, Zhang L. miR-487b promotes human umbilical vein endothelial cell proliferation, migration, invasion and tube formation through regulating THBS1. *Neurosci Lett* 2015;591:1–7.
241. Italiano A, Thomas R, Breen M, et al. The miR-17-92 cluster and its target THBS1 are differentially expressed in angiosarcomas dependent on MYC amplification. *Genes Chromosomes Cancer* 2012;51(6):569–578.
242. Li YX, Liu DQ, Zheng C, et al. miR-200a modulate HUVECs viability and migration. *IUBMB Life* 2011;63(7):553–559.
243. Muramatsu F, Kidoya H, Naito H, Sakimoto S, Takakura N. microRNA-125b inhibits tube formation of blood vessels through translational suppression of VE-cadherin. *Oncogene* 2013;32(4):414–421.
244. Zhao N, Sun H, Sun B, et al. miR-27a-3p suppresses tumor metastasis and VM by down-regulating VE-cadherin expression and inhibiting EMT: An essential role for Twist-1 in HCC. *Sci Rep* 2016;6:23091.
245. Zhang J, Han C, Zhu H, Song K, Wu T. miR-101 inhibits cholangiocarcinoma angiogenesis through targeting vascular endothelial growth factor (VEGF). *Am J Pathol* 2013;182(5):1629–1639.
246. Dai J, Wang J, Yang L, Xiao Y, Ruan Q. miR-125a regulates angiogenesis of gastric cancer by targeting vascular endothelial growth factor A. *Int J Oncol* 2015;47(5):1801–1810.
247. Chen H, Li L, Wang S, et al. Reduced miR-126 expression facilitates angiogenesis of gastric cancer through its regulation on VEGF-A. *Oncotarget* 2014;5(23):11873–11885.
248. Sasahira T, Kurihara M, Bhawal UK, et al. Downregulation of miR-126 induces angiogenesis and lymphangiogenesis by activation of VEGF-A in oral cancer. *Br J Cancer* 2012;107(4):700–706.
249. Zou C, Xu Q, Mao F, et al. MiR-145 inhibits tumor angiogenesis and growth by N-RAS and VEGF. *Cell Cycle* 2012;11(11):2137–2145.
250. Sun CY, She XM, Qin Y, et al. miR-15a and miR-16 affect the angiogenesis of multiple myeloma by targeting VEGF. *Carcinogenesis* 2013;34(2):426–435.
251. Hao Y, Yang J, Yin S, et al. The synergistic regulation of VEGF-mediated angiogenesis through miR-190 and target genes. *RNA* 2014;20(8):1328–1336.
252. Geng L, Chaudhuri A, Talmon G, et al. MicroRNA-192 suppresses liver metastasis of colon cancer. *Oncogene* 2014;33(46):5332–5340.

253. Wang R, Zhao N, Li S, et al. MicroRNA-195 suppresses angiogenesis and metastasis of hepatocellular carcinoma by inhibiting the expression of VEGF, VAV2, and CDC42. *Hepatology* 2013;58(2):642–653.
254. Choi YC, Yoon S, Jeong Y, Yoon J, Baek K. Regulation of vascular endothelial growth factor signaling by miR-200b. *Mol Cells* 2011;32(1):77–82.
255. Chou J, Lin JH, Brenot A, Kim JW, Provot S, Werb Z. GATA3 suppresses metastasis and modulates the tumour microenvironment by regulating microRNA-29b expression. *Nat Cell Biol* 2013;15(2):201–213.
256. Yan JJ, Zhang YN, Liao JZ, et al. MiR-497 suppresses angiogenesis and metastasis of hepatocellular carcinoma by inhibiting VEGFA and AEG-1. *Oncotarget* 2015;6(30):29527–29542.
257. Cheng J, Chen Y, Zhao P, et al. Downregulation of miRNA-638 promotes angiogenesis and growth of hepatocellular carcinoma by targeting VEGF. *Oncotarget* 2016;7(21):30702–30711.
258. Shi L, Zhang S, Wu H, et al. MiR-200c increases the radiosensitivity of non-small-cell lung cancer cell line A549 by targeting VEGF-VEGFR2 pathway. *PLoS One* 2013;8(10):e78344.
259. Park JK, Peng H, Yang W, Katsnelson J, Volpert O, Lavker RM. miR-184 exhibits angiostatic properties via regulation of Akt and VEGF signaling pathways. *FASEB J* 2017;31(1):256–265.
260. Wang S, Aurora AB, Johnson BA, et al. The endothelial-specific microRNA miR-126 governs vascular integrity and angiogenesis. *Dev Cell* 2008;15(2):261–271.
261. Fish JE, Santoro MM, Morton SU, et al. miR-126 regulates angiogenic signaling and vascular integrity. *Dev Cell* 2008;15(2):272–284.
262. Ebrahimi F, Gopalan V, Smith RA, Lam AK. miR-126 in human cancers: Clinical roles and current perspectives. *Exp Mol Pathol* 2014;96(1):98–107.
263. Liu B, Peng XC, Zheng XL, Wang J, Qin YW. MiR-126 restoration down-regulate VEGF and inhibit the growth of lung cancer cell lines in vitro and in vivo. *Lung Cancer* 2009;66(2):169–175.
264. Zhu N, Zhang D, Xie H, et al. Endothelial-specific intron-derived miR-126 is down-regulated in human breast cancer and targets both VEGFA and PIK3R2. *Mol Cell Biochem* 2011;351(1–2):157–164.
265. Zhang Y, Wang X, Xu B, et al. Epigenetic silencing of miR-126 contributes to tumor invasion and angiogenesis in colorectal cancer. *Oncol Rep* 2013;30(4):1976–1984.
266. Zhang Y, Yang P, Sun T, et al. miR-126 and miR-126* repress recruitment of mesenchymal stem cells and inflammatory monocytes to inhibit breast cancer metastasis. *Nat Cell Biol* 2013;15(3):284–294.
267. Png KJ, Halberg N, Yoshida M, et al. A microRNA regulon that mediates endothelial recruitment and metastasis by cancer cells. *Nature* 2011;481(7380):190–194.
268. Dews M, Homayouni A, Yu D, et al. Augmentation of tumor angiogenesis by a Myc-activated microRNA cluster. *Nat Genet* 2006;38(9):1060–1065.
269. Mendell JT. miRiad roles for the miR-17-92 cluster in development and disease. *Cell* 2008;133(2):217–222.
270. Volinia S, Calin GA, Liu CG, et al. A microRNA expression signature of human solid tumors defines cancer gene targets. *Proc Natl Acad Sci USA* 2006;103(7):2257–2261.
271. Chamorro-Jorganes A, Lee MY, Araldi E, et al. VEGF-induced expression of miR-17-92 cluster in endothelial cells is mediated by ERK/ELK1 activation and regulates angiogenesis. *Circ Res* 2016;118(1):38–47.
272. Doebele C, Bonauer A, Fischer A, et al. Members of the microRNA-17-92 cluster exhibit a cell-intrinsic antiangiogenic function in endothelial cells. *Blood* 2010;115(23):4944–4950.
273. Huang X, Ding L, Bennewith KL, et al. Hypoxia-inducible mir-210 regulates normoxic gene expression involved in tumor initiation. *Mol Cell* 2009;35(6):856–867.
274. Dang K, Myers KA. The role of hypoxia-induced miR-210 in cancer progression. *Int J Mol Sci* 2015;16(3):6353–6372.
275. Ivan M, Huang X. miR-210: Fine-tuning the hypoxic response. *Adv Exp Med Biol* 2014;772:205–227.
276. Borges NM, do Vale Elias M, Fook-Alves VL, et al. Angiomirs expression profiling in diffuse large B-cell lymphoma. *Oncotarget* 2016;7(4):4806–4816.
277. De Bock K, Georgiadou M, Schoors S, et al. Role of PFKFB3-driven glycolysis in vessel sprouting. *Cell* 2013;154(3):651–663.
278. Schoors S, De Bock K, Cantelmo AR,et al. Partial and transient reduction of glycolysis by PFKFB3 blockade reduces pathological angiogenesis. *Cell Metab* 2014;19(1):37–48.
279. Leopold JA, Zhang YY, Scribner AW, Stanton RC, Loscalzo J. Glucose-6-phosphate dehydrogenase overexpression decreases endothelial cell oxidant stress and increases bioavailable nitric oxide. *Arterioscler Thromb Vasc Biol* 2003;23(3):411–417.

280. Benedito R, Roca C, Sorensen I, et al. The notch ligands Dll4 and Jagged1 have opposing effects on angiogenesis. *Cell* 2009;137(6):1124–1135.
281. Croci DO, Cerliani JP, Dalotto-Moreno T, et al. Glycosylation-dependent lectin-receptor interactions preserve angiogenesis in anti-VEGF refractory tumors. *Cell* 2014;156(4):744–758.
282. Boon L, Ugarte-Berzal E, Vandooren J, Opdenakker G. Glycosylation of matrix metalloproteases and tissue inhibitors: present state, challenges and opportunities. *Biochem J* 2016;473(11):1471–1482.
283. Vizan P, Sanchez-Tena S, Alcarraz-Vizan G, et al. Characterization of the metabolic changes underlying growth factor angiogenic activation: Identification of new potential therapeutic targets. *Carcinogenesis* 2009;30(6):946–952.
284. Zhang Z, Apse K, Pang J, Stanton RC. High glucose inhibits glucose-6-phosphate dehydrogenase via cAMP in aortic endothelial cells. *J Biol Chem* 2000;275(51):40042–40047.
285. Stanton RC. Glucose-6-phosphate dehydrogenase, NADPH, and cell survival. *IUBMB Life* 2012;64(5):362–369.
286. Wilhelm K, Happel K, Eelen G, et al. FOXO1 couples metabolic activity and growth state in the vascular endothelium. *Nature* 2016;529(7585):216–220.
287. Glatz JF, Luiken JJ, Bonen A. Membrane fatty acid transporters as regulators of lipid metabolism: Implications for metabolic disease. *Physiol Rev* 2010;90(1):367–417.
288. Elmasri H, Ghelfi E, Yu CW, et al. Endothelial cell-fatty acid binding protein 4 promotes angiogenesis: Role of stem cell factor/c-kit pathway. *Angiogenesis* 2012;15(3):457–468.
289. Schoors S, Bruning U, Missiaen R, et al. Fatty acid carbon is essential for dNTP synthesis in endothelial cells. *Nature* 2015;520(7546):192–197.
290. Wenes M, Shang M, Di Matteo M, et al. Macrophage metabolism controls tumor blood vessel morphogenesis and metastasis. *Cell Metab* 2016;24(5):701–715.
291. Vasudev NS, Reynolds AR. Anti-angiogenic therapy for cancer: Current progress, unresolved questions and future directions. *Angiogenesis* 2014;17(3):471–494.
292. Casanovas O, Hicklin DJ, Bergers G, Hanahan D. Drug resistance by evasion of antiangiogenic targeting of VEGF signaling in late-stage pancreatic islet tumors. *Cancer Cell* 2005;8(4):299–309.
293. Batchelor TT, Sorensen AG, di Tomaso E, et al. AZD2171, a pan-VEGF receptor tyrosine kinase inhibitor, normalizes tumor vasculature and alleviates edema in glioblastoma patients. *Cancer Cell* 2007;11(1):83–95.
294. Crawford Y, Kasman I, Yu L, et al. PDGF-C mediates the angiogenic and tumorigenic properties of fibroblasts associated with tumors refractory to anti-VEGF treatment. *Cancer Cell* 2009;15(1):21–34.
295. Porta C, Paglino C, Imarisio I, et al. Changes in circulating pro-angiogenic cytokines, other than VEGF, before progression to sunitinib therapy in advanced renal cell carcinoma patients. *Oncology* 2013;84(2):115–122.
296. Llovet JM, Decaens T, Raoul JL, et al. Brivanib in patients with advanced hepatocellular carcinoma who were intolerant to sorafenib or for whom sorafenib failed: Results from the randomized phase III BRISK-PS study. *J Clin Oncol* 2013;31(28):3509–3516.
297. Johnson PJ, Qin S, Park JW, et al. Brivanib versus sorafenib as first-line therapy in patients with unresectable, advanced hepatocellular carcinoma: Results from the randomized phase III BRISK-FL study. *J Clin Oncol* 2013;31(28):3517–3524.
298. Liang WC, Wu X, Peale FV, et al. Cross-species vascular endothelial growth factor (VEGF)-blocking antibodies completely inhibit the growth of human tumor xenografts and measure the contribution of stromal VEGF. *J Biol Chem* 2006;281(2):951–961.
299. Liang W, Ferrara N. The complex role of neutrophils in tumor angiogenesis and metastasis. *Cancer Immunol Res* 2016;4(2):83–91.
300. Liang J, Piao Y, Holmes L, et al. Neutrophils promote the malignant glioma phenotype through S100A4. *Clin Cancer Res* 2014;20(1):187–198.
301. Botta C, Barbieri V, Ciliberto D, et al. Systemic inflammatory status at baseline predicts bevacizumab benefit in advanced non-small cell lung cancer patients. *Cancer Biol Ther* 2013;14(6):469–475.
302. Zhang QW, Liu L, Gong CY, et al. Prognostic significance of tumor-associated macrophages in solid tumor: A meta-analysis of the literature. *PLoS One* 2012;7(12):e50946.
303. Coffelt SB, Lewis CE, Naldini L, Brown JM, Ferrara N, De Palma M. Elusive identities and overlapping phenotypes of proangiogenic myeloid cells in tumors. *Am J Pathol* 2010;176(4):1564–1576.
304. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. *Nature* 2008;454(7203):436–444.

305. Kim J, Bae JS. Tumor-associated macrophages and neutrophils in tumor microenvironment. *Mediators Inflamm* 2016;2016:6058147.
306. Chung AS, Wu X, Zhuang G, et al. An interleukin-17-mediated paracrine network promotes tumor resistance to anti-angiogenic therapy. *Nat Med* 2013;19(9):1114–1123.
307. Bailey AS, Willenbring H, Jiang S, et al. Myeloid lineage progenitors give rise to vascular endothelium. *Proc Natl Acad Sci USA* 2006;103(35):13156–13161.
308. Dome B, Hendrix MJ, Paku S, Tovari J, Timar J. Alternative vascularization mechanisms in cancer: Pathology and therapeutic implications. *Am J Pathol* 2007;170(1):1–15.
309. Hlushchuk R, Riesterer O, Baum O, et al. Tumor recovery by angiogenic switch from sprouting to intussusceptive angiogenesis after treatment with PTK787/ZK222584 or ionizing radiation. *Am J Pathol* 2008;173(4):1173–1185.
310. Mancuso MR, Davis R, Norberg SM, et al. Rapid vascular regrowth in tumors after reversal of VEGF inhibition. *J Clin Invest* 2006;116(10):2610–2621.
311. Semela D, Piguet AC, Kolev M, et al. Vascular remodeling and antitumoral effects of mTOR inhibition in a rat model of hepatocellular carcinoma. *J Hepatol* 2007;46(5):840–848.
312. Drevs J, Muller-Driver R, Wittig C, et al. PTK787/ZK 222584, a specific vascular endothelial growth factor-receptor tyrosine kinase inhibitor, affects the anatomy of the tumor vascular bed and the functional vascular properties as detected by dynamic enhanced magnetic resonance imaging. *Cancer Res* 2002;62(14):4015–4022.
313. Nakamura K, Yamamoto A, Kamishohara M, et al. KRN633: A selective inhibitor of vascular endothelial growth factor receptor-2 tyrosine kinase that suppresses tumor angiogenesis and growth. *Mol Cancer Ther* 2004;3(12):1639–1649.
314. Nakamura K, Taguchi E, Miura T, et al. KRN951, a highly potent inhibitor of vascular endothelial growth factor receptor tyrosine kinases, has antitumor activities and affects functional vascular properties. *Cancer Res* 2006;66(18):9134–9142.
315. Ruggeri B, Singh J, Gingrich D, et al. CEP-7055: A novel, orally active pan inhibitor of vascular endothelial growth factor receptor tyrosine kinases with potent antiangiogenic activity and antitumor efficacy in preclinical models. *Cancer Res* 2003;63(18):5978–5991.
316. Rudin M, McSheehy PM, Allegrini PR, et al. PTK787/ZK222584, a tyrosine kinase inhibitor of vascular endothelial growth factor receptor, reduces uptake of the contrast agent GdDOTA by murine orthotopic B16/BL6 melanoma tumours and inhibits their growth in vivo. *NMR Biomed* 2005;18(5):308–321.
317. Leenders WP, Kusters B, Verrijp K, et al. Antiangiogenic therapy of cerebral melanoma metastases results in sustained tumor progression via vessel co-option. *Clin Cancer Res* 2004;10(18 Pt 1):6222–6230.
318. Rubenstein JL, Kim J, Ozawa T, et al. Anti-VEGF antibody treatment of glioblastoma prolongs survival but results in increased vascular cooption. *Neoplasia* 2000;2(4):306–314.
319. Kuczynski EA, Yin M, Bar-Zion A, et al. Co-option of liver vessels and not sprouting angiogenesis drives acquired sorafenib resistance in hepatocellular carcinoma. *J Natl Cancer Inst* 2016;108(8).
320. Helfrich I, Scheffrahn I, Bartling S, et al. Resistance to antiangiogenic therapy is directed by vascular phenotype, vessel stabilization, and maturation in malignant melanoma. *J Exp Med* 2010;207(3):491–503.
321. Frentzas S, Simoneau E, Bridgeman VL, et al. Vessel co-option mediates resistance to anti-angiogenic therapy in liver metastases. *Nat Med* 2016;22(11):1294–1302.
322. Yu JL, Coomber BL, Kerbel RS. A paradigm for therapy-induced microenvironmental changes in solid tumors leading to drug resistance. *Differentiation* 2002;70(9–10):599–609.
323. Yu JL, Rak JW, Carmeliet P, Nagy A, Kerbel RS, Coomber BL. Heterogeneous vascular dependence of tumor cell populations. *Am J Pathol* 2001;158(4):1325–1334.
324. Yu JL, Rak JW, Coomber BL, Hicklin DJ, Kerbel RS. Effect of p53 status on tumor response to antiangiogenic therapy. *Science* 2002;295(5559):1526–1528.
325. Pisarsky L, Bill R, Fagiani E, et al. Targeting metabolic symbiosis to overcome resistance to anti-angiogenic therapy. *Cell Rep* 2016;15(6):1161–1174.
326. Semenza GL. Defining the role of hypoxia-inducible factor 1 in cancer biology and therapeutics. *Oncogene* 2010;29(5):625–634.
327. Abraham J, Salama NN, Azab AK. The role of P-glycoprotein in drug resistance in multiple myeloma. *Leuk Lymphoma* 2015;56(1):26–33.
328. Gray LH, Conger AD, Ebert M, Hornsey S, Scott OC. The concentration of oxygen dissolved in tissues at the time of irradiation as a factor in radiotherapy. *Br J Radiol* 1953;26(312):638–648.

329. Marie-Egyptienne DT, Lohse I, Hill RP. Cancer stem cells, the epithelial to mesenchymal transition (EMT) and radioresistance: Potential role of hypoxia. *Cancer Lett* 2013;341(1):63–72.
330. Muz B, de la Puente P, Azab F, Luderer M, Azab AK. Hypoxia promotes stem cell-like phenotype in multiple myeloma cells. *Blood Cancer J* 2014;4:e262.
331. Rohwer N, Cramer T. Hypoxia-mediated drug resistance: Novel insights on the functional interaction of HIFs and cell death pathways. *Drug Resist Updat* 2011;14(3):191–201.
332. Kimura H, Braun RD, Ong ET, et al. Fluctuations in red cell flux in tumor microvessels can lead to transient hypoxia and reoxygenation in tumor parenchyma. *Cancer Res* 1996;56(23):5522–5528.
333. Pires IM, Bencokova Z, Milani M, et al. Effects of acute versus chronic hypoxia on DNA damage responses and genomic instability. *Cancer Res* 2010;70(3):925–935.
334. Semenza GL. Hypoxia-inducible factors in physiology and medicine. *Cell* 2012;148(3):399–408.
335. Ke Q, Costa M. Hypoxia-inducible factor-1 (HIF-1). *Mol Pharmacol* 2006;70(5):1469–1480.
336. Duran I, Lambea J, Maroto P, et al. Resistance to targeted therapies in renal cancer: The importance of changing the mechanism of action. *Target Oncol* 2017;12(1):19–35.
337. Kilic-Eren M, Boylu T, Tabor V. Targeting PI3K/Akt represses hypoxia inducible factor-1alpha activation and sensitizes Rhabdomyosarcoma and Ewing's sarcoma cells for apoptosis. *Cancer Cell Int* 2013;13:36.
338. Tafani M, Pucci B, Russo A, et al. Modulators of HIF1alpha and NFkB in cancer treatment: Is it a rational approach for controlling malignant progression? *Front Pharmacol* 2013;4:13.
339. de la Puente P, Muz B, Azab F, Azab AK. Cell trafficking of endothelial progenitor cells in tumor progression. *Clin Cancer Res* 2013;19(13):3360–3368.
340. Krock BL, Skuli N, Simon MC. Hypoxia-induced angiogenesis: Good and evil. *Genes Cancer* 2011;2(12):1117–1133.
341. Muz B, de la Puente P, Azab F, Azab AK. The role of hypoxia in cancer progression, angiogenesis, metastasis, and resistance to therapy. *Hypoxia (Auckl)* 2015;3:83–92.
342. Ranpura V, Hapani S, Wu S. Treatment-related mortality with bevacizumab in cancer patients: A meta-analysis. *JAMA* 2011;305(5):487–494.
343. Schutz FA, Je Y, Richards CJ, Choueiri TK. Meta-analysis of randomized controlled trials for the incidence and risk of treatment-related mortality in patients with cancer treated with vascular endothelial growth factor tyrosine kinase inhibitors. *J Clin Oncol* 2012;30(8):871–877.
344. Qi WX, Tang LN, Sun YJ, et al. Incidence and risk of hemorrhagic events with vascular endothelial growth factor receptor tyrosine-kinase inhibitors: An up-to-date meta-analysis of 27 randomized controlled trials. *Ann Oncol* 2013;24(12):2943–2952.
345. Zhang ZF, Wang T, Liu LH, Guo HQ. Risks of proteinuria associated with vascular endothelial growth factor receptor tyrosine kinase inhibitors in cancer patients: A systematic review and meta-analysis. *PLoS One* 2014;9(3):e90135.
346. Kouri BE, Funaki BS, Ray CE, Jr, et al. ACR appropriateness criteria radiologic management of hepatic malignancy. *J Am Coll Radiol* 2012;9(12):919–925.
347. van Erp NP, Mathijssen RH, van der Veldt AA, et al. Myelosuppression by sunitinib is flt-3 genotype dependent. *Br J Cancer* 2010;103(5):757–758.
348. Feldt S, Schussel K, Quinzler R, et al. Incidence of thyroid hormone therapy in patients treated with sunitinib or sorafenib: A cohort study. *Eur J Cancer* 2012;48(7):974–981.
349. Kruser TJ, Wheeler DL. Mechanisms of resistance to HER family targeting antibodies. *Exp Cell Res* 2010;316(7):1083–1100.
350. Van Cutsem E, Tabernero J, Lakomy R, et al. Addition of aflibercept to fluorouracil, leucovorin, and irinotecan improves survival in a phase III randomized trial in patients with metastatic colorectal cancer previously treated with an oxaliplatin-based regimen. *J Clin Oncol* 2012;30(28):3499–3506.
351. Motzer RJ, Hutson TE, Tomczak P, et al. Overall survival and updated results for sunitinib compared with interferon alfa in patients with metastatic renal cell carcinoma. *J Clin Oncol* 2009;27(22):3584–3590.
352. Limaverde-Sousa G, Sternberg C, Ferreira CG. Antiangiogenesis beyond VEGF inhibition: A journey from antiangiogenic single-target to broad-spectrum agents. *Cancer Treat Rev* 2014;40(4):548–557.
353. Paz-Ares LG, Biesma B, Heigener D, et al. Group NREUSIS. Phase III, randomized, double-blind, placebo-controlled trial of gemcitabine/cisplatin alone or with sorafenib for the first-line treatment of advanced, nonsquamous non-small-cell lung cancer. *J Clin Oncol* 2012;30(25):3084–3092.

354. Ledermann JA, Hackshaw A, Kaye S, et al. Randomized phase II placebo-controlled trial of maintenance therapy using the oral triple angiokinase inhibitor BIBF 1120 after chemotherapy for relapsed ovarian cancer. *J Clin Oncol* 2011;29(28):3798–3804.
355. Torimura T, Iwamoto H, Nakamura T, et al. Antiangiogenic and antitumor activities of aflibercept, a soluble VEGF receptor-1 and -2, in a mouse model of hepatocellular carcinoma. *Neoplasia* 2016;18(7):413–424.
356. Lee JH, Canny MD, De Erkenez A, et al. A therapeutic aptamer inhibits angiogenesis by specifically targeting the heparin binding domain of VEGF165. *Proc Natl Acad Sci USA* 2005;102(52):18902–18907.
357. Kabbinavar F, Hurwitz HI, Fehrenbacher L, et al. Phase II, randomized trial comparing bevacizumab plus fluorouracil (FU)/leucovorin (LV) with FU/LV alone in patients with metastatic colorectal cancer. *J Clin Oncol* 2003;21(1):60–65.
358. Gaudreault J, Fei D, Rusit J, Suboc P, Shiu V. Preclinical pharmacokinetics of Ranibizumab (rhuFabV2) after a single intravitreal administration. *Invest Ophthalmol Vis Sci* 2005;46(2):726–733.
359. Sullivan LA, Carbon JG, Roland CL, et al. r84, a novel therapeutic antibody against mouse and human VEGF with potent anti-tumor activity and limited toxicity induction. *PLoS One* 2010;5(8):e12031.
360. Krupitskaya Y, Wakelee HA. Ramucirumab, a fully human mAb to the transmembrane signaling tyrosine kinase VEGFR-2 for the potential treatment of cancer. *Curr Opin Investig Drugs* 2009;10(6):597–605.
361. LoRusso PM, Krishnamurthi S, Youssoufian H, et al. Icrucumab, a fully human monoclonal antibody against the vascular endothelial growth factor receptor-1, in the treatment of patients with advanced solid malignancies: A Phase 1 study. *Invest New Drugs* 2014;32(2):303–311.
362. Harding TC, Long L, Palencia S, et al. Blockade of nonhormonal fibroblast growth factors by FP-1039 inhibits growth of multiple types of cancer. *Sci Transl Med* 2013;5(178):178ra139.
363. Ronca R, Giacomini A, Di Salle E, et al. Long-pentraxin 3 derivative as a small-molecule FGF trap for cancer therapy. *Cancer Cell* 2015;28(2):225–239.
364. Bono F, De Smet F, Herbert C, et al. Inhibition of tumor angiogenesis and growth by a small-molecule multi-FGF receptor blocker with allosteric properties. *Cancer Cell* 2013;23(4):477–488.
365. Gavine PR, Mooney L, Kilgour E, et al. AZD4547: an orally bioavailable, potent, and selective inhibitor of the fibroblast growth factor receptor tyrosine kinase family. *Cancer Res* 2012;72(8):2045–2056.
366. Guagnano V, Furet P, Spanka C, et al. Discovery of 3-(2,6-dichloro-3,5-dimethoxy-phenyl)-1-{6-[4-(4-ethyl-piperazin-1-yl)-phenylamin o]-pyrimidin-4-yl}-1-methyl-urea (NVP-BGJ398), a potent and selective inhibitor of the fibroblast growth factor receptor family of receptor tyrosine kinase. *J Med Chem* 2011;54(20):7066–7083.
367. Zhao G, Li WY, Chen D, et al. A novel, selective inhibitor of fibroblast growth factor receptors that shows a potent broad spectrum of antitumor activity in several tumor xenograft models. *Mol Cancer Ther* 2011;10(11):2200–2210.
368. Mita AC, Takimoto CH, Mita M, et al. Phase 1 study of AMG 386, a selective angiopoietin 1/2-neutralizing peptibody, in combination with chemotherapy in adults with advanced solid tumors. *Clin Cancer Res* 2010;16(11):3044–3056.
369. Papadopoulos KP, Kelley RK, Tolcher AW, et al. A phase I first-in-human study of nesvacumab (REGN910), a fully human anti-angiopoietin-2 (Ang2) monoclonal antibody, in patients with advanced solid tumors. *Clin Cancer Res* 2016;22(6):1348–1355.
370. Peterson TE, Kirkpatrick ND, Huang Y, et al. Dual inhibition of Ang-2 and VEGF receptors normalizes tumor vasculature and prolongs survival in glioblastoma by altering macrophages. *Proc Natl Acad Sci USA* 2016;113(16):4470–4475.
371. Stubenrauch K, Wessels U, Essig U, et al. An immunodepletion procedure advances free angiopoietin-2 determination in human plasma samples during anti-cancer therapy with bispecific anti-Ang2/VEGF CrossMab. *J Pharm Biomed Anal* 2015;102:459–467.
372. Pecen PE, Kaiser PK. Current phase 1/2 research for neovascular age-related macular degeneration. *Curr Opin Ophthalmol* 2015;26(3):188–193.
373. Wood JM, Bold G, Buchdunger E, et al. PTK787/ZK 222584, a novel and potent inhibitor of vascular endothelial growth factor receptor tyrosine kinases, impairs vascular endothelial growth factor-induced responses and tumor growth after oral administration. *Cancer Res* 2000;60(8):2178–2189.
374. Kumar R, Knick VB, Rudolph SK, et al. Pharmacokinetic-pharmacodynamic correlation from mouse to human with pazopanib, a multikinase angiogenesis inhibitor with potent antitumor and antiangiogenic activity. *Mol Cancer Ther* 2007;6(7):2012–2021.
375. Wilhelm S, Chien DS. BAY 43–9006: Preclinical data. *Curr Pharm Des* 2002;8(25):2255–2257.
376. O'Farrell AM, Abrams TJ, Yuen HA, et al. SU11248 is a novel FLT3 tyrosine kinase inhibitor with potent activity in vitro and in vivo. *Blood* 2003;101(9):3597–3605.

377. Rugo HS, Herbst RS, Liu G, et al. Phase I trial of the oral antiangiogenesis agent AG-013736 in patients with advanced solid tumors: Pharmacokinetic and clinical results. *J Clin Oncol* 2005;23(24):5474–5483.

378. O'Hare T, Shakespeare WC, Zhu X, et al. AP24534, a pan-BCR-ABL inhibitor for chronic myeloid leukemia, potently inhibits the T315I mutant and overcomes mutation-based resistance. *Cancer Cell* 2009;16(5):401–412.

379. Wilhelm SM, Dumas J, Adnane L, et al. Regorafenib (BAY 73–4506): A new oral multikinase inhibitor of angiogenic, stromal and oncogenic receptor tyrosine kinases with potent preclinical antitumor activity. *Int J Cancer* 2011;129(1):245–255.

380. Houvras Y, Wirth LJ. Cabozantinib in medullary thyroid carcinoma: Time to focus the spotlight on this rare disease. *J Clin Oncol* 2011;29(19):2616–2618.

381. Wedge SR, Ogilvie DJ, Dukes M, et al. ZD6474 inhibits vascular endothelial growth factor signaling, angiogenesis, and tumor growth following oral administration. *Cancer Res* 2002;62(16):4645–4655.

382. Matsui J, Funahashi Y, Uenaka T, Watanabe T, Tsuruoka A, Asada M. Multi-kinase inhibitor E7080 suppresses lymph node and lung metastases of human mammary breast tumor MDA-MB-231 via inhibition of vascular endothelial growth factor-receptor (VEGF-R) 2 and VEGF-R3 kinase. *Clin Cancer Res* 2008;14(17):5459–5465.

383. Hilberg F, Roth GJ, Krssak M, et al. BIBF 1120: Triple angiokinase inhibitor with sustained receptor blockade and good antitumor efficacy. *Cancer Res* 2008;68(12):4774–4782.

384. Eikesdal HP, Sugimoto H, Birrane G, et al. Identification of amino acids essential for the antiangiogenic activity of tumstatin and its use in combination antitumor activity. *Proc Natl Acad Sci USA* 2008;105(39):15040–15045.

385. Perez-Perez MJ, Priego EM, Bueno O, Martins MS, Canela MD, Liekens S. Blocking blood flow to solid tumors by destabilizing tubulin: An approach to targeting tumor growth. *J Med Chem* 2016;59(19):8685–8711.

386. Monk BJ, Sill MW, Walker JL, et al. Randomized phase II evaluation of bevacizumab versus bevacizumab plus fosbretabulin in recurrent ovarian, tubal, or peritoneal carcinoma: An NRG Oncology/Gynecologic Oncology Group Study. *J Clin Oncol* 2016;34(19):2279–2286.

387. Kandalaft LE, Motz GT, Busch J, Coukos G. Angiogenesis and the tumor vasculature as antitumor immune modulators: The role of vascular endothelial growth factor and endothelin. *Curr Top Microbiol Immunol* 2011;344:129–148.

388. Voron T, Colussi O, Marcheteau E, et al. VEGF-A modulates expression of inhibitory checkpoints on $CD8^+$ T cells in tumors. *J Exp Med* 2015;212(2):139–148.

389. Shrimali RK, Yu Z, Theoret MR, Chinnasamy D, Restifo NP, Rosenberg SA. Antiangiogenic agents can increase lymphocyte infiltration into tumor and enhance the effectiveness of adoptive immunotherapy of cancer. *Cancer Res* 2010;70(15):6171–6180.

390. Hodi FS, Lawrence D, Lezcano C, et al. Bevacizumab plus ipilimumab in patients with metastatic melanoma. *Cancer Immunol Res* 2014;2(7):632–642.

391. Ott PA, Hodi FS, Buchbinder EI. Inhibition of immune checkpoints and vascular endothelial growth factor as combination therapy for metastatic melanoma: An overview of rationale, preclinical evidence, and initial clinical data. *Front Oncol* 2015;5:202.

392. Izumi Y, Xu L, di Tomaso E, Fukumura D, Jain RK. Tumour biology: Herceptin acts as an anti-angiogenic cocktail. *Nature* 2002;416(6878):279–280.

393. Tolaney SM, Boucher Y, Duda DG, et al. Role of vascular density and normalization in response to neoadjuvant bevacizumab and chemotherapy in breast cancer patients. *Proc Natl Acad Sci USA* 2015;112(46):14325–14330.

394. Park JS, Kim IK, Han S, et al. Normalization of tumor vessels by Tie2 activation and Ang2 inhibition enhances drug delivery and produces a favorable tumor microenvironment. *Cancer Cell* 2016;30(6):953–967.

395. Peterson TE, Kirkpatrick ND, Huang Y, et al. Dual inhibition of Ang-2 and VEGF receptors normalizes tumor vasculature and prolongs survival in glioblastoma by altering macrophages. *Proc Natl Acad Sci USA* 2016;113(16):4470–4475.

396. Huang M, Liu T, Ma P, et al. c-Met-mediated endothelial plasticity drives aberrant vascularization and chemoresistance in glioblastoma. *J Clin Invest* 2016;126(5):1801–1814.

397. Chauhan VP, Stylianopoulos T, Martin JD, et al. Normalization of tumour blood vessels improves the delivery of nanomedicines in a size-dependent manner. *Nat Nanotechnol* 2012;7(6):383–388.

398. Huang Y, Yuan J, Righi E, et al. Vascular normalizing doses of antiangiogenic treatment reprogram the immunosuppressive tumor microenvironment and enhance immunotherapy. *Proc Natl Acad Sci USA* 2012;109(43):17561–17566.

399. Jain RK, Safabakhsh N, Sckell A, et al. Endothelial cell death, angiogenesis, and microvascular function after castration in an androgen-dependent tumor: Role of vascular endothelial growth factor. *Proc Natl Acad Sci USA* 1998;95(18):10820–10825.
400. Bottos A, Martini M, Di Nicolantonio F, et al. Targeting oncogenic serine/threonine-protein kinase BRAF in cancer cells inhibits angiogenesis and abrogates hypoxia. *Proc Natl Acad Sci USA* 2012;109(6):E353–E359.
401. Maes H, Kuchnio A, Peric A, et al. Tumor vessel normalization by chloroquine independent of autophagy. *Cancer Cell* 2014;26(2):190–206.
402. Goel S, Duda DG, Xu L, et al. Normalization of the vasculature for treatment of cancer and other diseases. *Physiol Rev* 2011;91(3):1071–1121.
403. Huang Y, Stylianopoulos T, Duda DG, Fukumura D, Jain RK. Benefits of vascular normalization are dose and time dependent–letter. *Cancer Res* 2013;73(23):7144–7146.
404. Giacomini A, Chiodelli P, Matarazzo S, Rusnati M, Presta M, Ronca R. Blocking the FGF/FGFR system as a "two-compartment" antiangiogenic/antitumor approach in cancer therapy. *Pharmacol Res* 2016;107:172–185.
405. Ronca R, Giacomini A, Rusnati M, Presta M. The potential of fibroblast growth factor/fibroblast growth factor receptor signaling as a therapeutic target in tumor angiogenesis. *Expert Opin Ther Targets* 2015;19(10):1361–1377.
406. Dieci MV, Arnedos M, Andre F, Soria JC. Fibroblast growth factor receptor inhibitors as a cancer treatment: From a biologic rationale to medical perspectives. *Cancer Discov* 2013;3(3):264–279.
407. Zhao Y, Adjei AA. Targeting angiogenesis in cancer therapy: Moving beyond vascular endothelial growth factor. *Oncologist* 2015;20(6):660–673.
408. Aprile G, Macerelli M, Giuliani F. Regorafenib for gastrointestinal malignancies: From preclinical data to clinical results of a novel multi-target inhibitor. *BioDrugs* 2013;27(3):213–224.
409. Hilberg F, Roth GJ, Krssak M, et al. BIBF 1120: Triple angiokinase inhibitor with sustained receptor blockade and good antitumor efficacy. *Cancer Res* 2008;68(12):4774–4782.
410. Burroughs SK, Kaluz S, Wang D, Wang K, Van Meir EG, Wang B. Hypoxia inducible factor pathway inhibitors as anticancer therapeutics. *Future Med Chem* 2013;5(5):553–572.
411. Subhani S, Vavilala DT, Mukherji M. HIF inhibitors for ischemic retinopathies and cancers: Options beyond anti-VEGF therapies. *Angiogenesis* 2016;19(3):257–273.
412. Viziteu E, Grandmougin C, Goldschmidt H, et al. Chetomin, targeting HIF-1alpha/p300 complex, exhibits antitumour activity in multiple myeloma. *Br J Cancer* 2016;114(5):519–523.
413. Greenberger LM, Horak ID, Filpula D, et al. A RNA antagonist of hypoxia-inducible factor-1alpha, EZN-2968, inhibits tumor cell growth. *Mol Cancer Ther* 2008;7(11):3598–3608.
414. Kim YH, Coon A, Baker AF, Powis G. Antitumor agent PX-12 inhibits HIF-1alpha protein levels through an Nrf2/PMF-1-mediated increase in spermidine/spermine acetyl transferase. *Cancer Chemother Pharmacol* 2011;68(2):405–413.
415. Ramanathan RK, Stephenson JJ, Weiss GJ, et al. A phase I trial of PX-12, a small-molecule inhibitor of thioredoxin-1, administered as a 72-hour infusion every 21 days in patients with advanced cancers refractory to standard therapy. *Invest New Drugs* 2012;30(4):1591–1596.
416. Lin J, Zhan T, Duffy D, et al. A pilot phase II study of digoxin in patients with recurrent prostate cancer as evident by a rising PSA. *Am J Cancer Ther Pharmacol* 2014;2(1):21–32.
417. Gayed BA, O'Malley KJ, Pilch J, Wang Z. Digoxin inhibits blood vessel density and HIF-1a expression in castration-resistant C4-2 xenograft prostate tumors. *Clin Transl Sci* 2012;5(1):39–42.
418. Neri D, Bicknell R. Tumour vascular targeting. *Nat Rev Cancer* 2005;5(6):436–446.
419. Bootz F, Neri D. Immunocytokines: A novel class of products for the treatment of chronic inflammation and autoimmune conditions. *Drug Discov Today* 2016;21(1):180–189.
420. Ronca R, Sozzani S, Presta M, Alessi P. Delivering cytokines at tumor site: The immunocytokine-conjugated anti-EDB-fibronectin antibody case. *Immunobiology* 2009;214(9–10):800–810.
421. Rybak JN, Ettorre A, Kaissling B, Giavazzi R, Neri D, Elia G. In vivo protein biotinylation for identification of organ-specific antigens accessible from the vasculature. *Nat Methods* 2005;2(4):291–298.
422. Borgia B, Roesli C, Fugmann T, et al. A proteomic approach for the identification of vascular markers of liver metastasis. *Cancer Res* 2010;70(1):309–318.
423. Frey K, Fiechter M, Schwager K, et al. Different patterns of fibronectin and tenascin-C splice variants expression in primary and metastatic melanoma lesions. *Exp Dermatol* 2011;20(8):685–688.

424. Schwager K, Bootz F, Imesch P, Kaspar M, Trachsel E, Neri D. The antibody-mediated targeted delivery of interleukin-10 inhibits endometriosis in a syngeneic mouse model. *Hum Reprod* 2011;26(9):2344–2352.
425. Schliemann C, Roesli C, Kamada H, et al. In vivo biotinylation of the vasculature in B-cell lymphoma identifies BST-2 as a target for antibody-based therapy. *Blood* 2010;115(3):736–744.
426. Neri D, Sondel PM. Immunocytokines for cancer treatment: Past, present and future. *Curr Opin Immunol* 2016;40:96–102.
427. Treps L, Conradi LC, Harjes U, Carmeliet P. Manipulating angiogenesis by targeting endothelial metabolism: Hitting the engine rather than the drivers—A new perspective? *Pharmacol Rev* 2016;68(3):872–887.
428. Cantelmo AR, Conradi LC, Brajic A, et al. Inhibition of the glycolytic activator PFKFB3 in endothelium induces tumor vessel normalization, impairs metastasis, and improves chemotherapy. *Cancer Cell* 2016;30(6):968–985.

AUTHOR'S BIOGRAPHIES

Roberto Ronca is assistant professor in General Pathology at the Department of Molecular and Translational Medicine at the University of Brescia. After graduating in Biology in 1999 at the University Padua (Italy), he obtained his PhD degree (2003) in Medical Biotechnology at the University of Brescia (Italy) on the role of FGF/FGFR system in vascular biology and in tumor angiogenesis. His research is currently focused on the study of tumor-stroma interactions and tumor growth to develop therapeutic approaches based both on decoy molecules and antibodies as anti-cancer and anti-angiogenetic agents.

Mohammed Benkheil graduated in Biomedical Science in 2012 at the University of Leuven, Belgium. Since then, he is enrolled in a PhD program at the Rega Institute (Laboratory of Virology and Chemotherapy, University of Leuven, Belgium). His project is on the role of hepatitis C virus (HCV) in liver cancer pathogenesis, with particular focus on tumor angiogenesis.

Stefania Mitola graduated in Biological Sciences in 1995 at the University of Torino, Italy. She obtained her Specialization (2001) in Clinical Chemistry and Biochemistry (University of Torino, Italy) on the interaction between VEGFR2 and integrins during angiogenesis. She is an associate professor in Biochemistry at the University of Brescia. Her research is currently focused on the characterization of pro-angiogenic molecules and on canonical and non canonical ligands of VEGFR2.

Sofie Struyf graduated as Bio-Engineer in 1996 at the University of Leuven, Belgium. She joined the Laboratory of Molecular Immunology at the Rega Institute (University of Leuven, Belgium) for her doctoral research on post-translational modifications of chemokines. On this subject she obtained her PhD degree in applied biological sciences in 2002. Since 2009 she is holding a position of professor at the Rega Institute. Her research is currently focused on the role of chemokines in angiogenesis and cancer.

Sandra Liekens graduated as Bio-Engineer in 1994 at the University of Leuven, Belgium. She obtained her PhD degree (2000) in Applied Biological Sciences at the Rega Institute (Laboratory of Virology and Chemotherapy, University of Leuven, Belgium) on the inhibition of vascular tumor growth by anti-angiogenic and apoptosis-inducing agents. She is holding a position of professor at the Rega Institute. Her research is currently focused on understanding the interplay between viral and cellular factors involved in angiogenesis and the characterization of anti-angiogenic and vascular disrupting agents.

How to cite this article: Ronca R, Benkheil M, Mitola S, Struyf S and Liekens S. Tumor angiogenesis revisited: Regulators and clinical implications. *Med Res Rev*. 2017;37:1231–1274. https://doi.org/10.1002/med.21452